RENAL FUNCTION

Transactions of the Fourth Conference
October 22, 23, and 24, 1952, New York, N. Y.

Edited by
STANLEY E BRADLEY, M D
ASSOCIATE PROFESSOR OF MEDICINE
COLUMBIA UNIVERSITY
COLLEGE OF PHYSICIANS AND SURGEONS
NEW YORK, N Y

Sponsored by the
JOSIAH MACY, JR FOUNDATION
NEW YORK, N Y

Library of Congress Catalog Card Number 51 33198
Price $3 50

Printed in the United States of America
By Corlies Macy & Company Inc New York, N Y

PARTICIPANTS

Fourth Conference on Renal Function

MEMBERS

ROBERT F PITTS *Chairman*
Department of Physiology Cornell University Medical College
New York N Y

STANLEY E BRADLEY, *Secretary*
Department of Medicine Columbia University College of Physicians and Surgeons
New York N Y

ROBERT W BERLINER
Section on Kidney and Electrolyte Metabolism National Heart Institute
National Institutes of Health
Bethesda Md

FRANCIS BINKLEY
Department of Biochemistry Emory University School of Dentistry
Emory University Ga

PHYLLIS A BOTT
Department of Physiological Chemistry Woman s Medical College of Pennsylvania
Philadelphia Pa

DANIEL C DARROW
Department of Pediatrics Yale University School of Medicine
New Haven Conn

WILLIAM DOCK
Department of Medicine College of Medicine State University of New York
Medical Center at New York City
Brooklyn N Y

ARTHUR M FISHBERG
Department of Medicine Beth Israel Hospital
New York N Y

ROY P FORSTER
Department of Zoology Dartmouth College
Hanover N H

ALLAN L GRAFFLIN*
Department of Anatomy Johns Hopkins University School of Medicine
Baltimore Md

JOHN A LUETSCHER JR
Department of Medicine Stanford University School of Medicine
San Francisco Calif

JEAN OLIVER
Department of Pathology College of Medicine State University of New York
Medical Center at New York City
Brooklyn N Y

EWALD E SELKURT
Department of Physiology Western Reserve University School of Medicine
Cleveland Ohio

JAMES A SHANNON*
National Heart Institute National Institutes of Health
Bethesda, Md

---

*Absent

NATHAN W SHOCK
Section on Gerontology National Heart Institute National Institutes of Health and Baltimore City Hospitals
Baltimore Md

JOHN V TAGGART
Department of Medicine Columbia University College of Physicians and Surgeons
New York, N Y

GEORGE W THORN
Department of Medicine Peter Bent Brigham Hospital
Boston Mass

GUESTS

KLAUS BRUN
Third Medical Department City Municipal Hospital
Copenhagen Denmark

ROBERT W CLARKE
Army Medical Service Graduate School Walter Reed Army Medical Center
Washington D C

J RUSSELL ELKINTON
Department of Medicine University of Pennsylvania School of Medicine
Philadelphia Pa

WILLIAM D LOTSPEICH
Department of Physiology University of Cincinnati College of Medicine
Cincinnati Ohio

GILBERT H MUDGE
Department of Medicine Columbia University College of Physicians and Surgeons
New York N Y

ROBERT PHILIPS *Comdr (MC) USN*
Bureau of Medicine and Surgery Navy Department
Washington D C

H BURR STEINBACH
Department of Zoology University of Minnesota
Minneapolis Minn

ROY C SWAN
Department of Physiology Cornell University Medical College
New York N Y

HANS H USSING
Department of Biological Isotope Research
Zoophysiological Laboratory The University of Copenhagen
Copenhagen Denmark

MAURICE B VISSCHER
Department of Physiology University of Minnesota Medical School
Minneapolis Minn

WILLIAM WALLACE
Department of Pediatrics Western Reserve University School of Medicine
Cleveland Ohio

Josiah Macy Jr Foundation
FRANK FREMONT SMITH, *Medical Director*
JANET FREED, *Assistant for the Conference Program*

TABLE OF CONTENTS

Fourth Conference on Renal Function

JOSIAH MACY, JR FOUNDATION CONFERENCE PROGRAM

As an introduction to these Transactions of the Fourth Conference on Renal Function I should like to outline what it is that the Foundation hopes to accomplish by its Conference Program We are interested first of all in furthering knowledge in a particular field i e renal function and to this end the participants were brought together to exchange ideas experiences data and methods In addition to this particular goal however there is a further and perhaps more fundamental aim which is shared by all our conference groups This is the promotion of meaningful communication between scientific disciplines

The problem of communication between disciplines we feel to be a very real and urgent one the most effective advancement of the whole of science being to a large extent dependent upon it Because of the accelerating rate at which new knowledge is accumulating and because discoveries in one field so often result from information gained in quite another channels must be established for the most effective dissemination and exchange of this knowledge

The increasing realization that nature itself recognizes no boundaries makes it evident that the continued isolation of the several branches of science is a serious obstacle to scientific progress Particularly is it true in medicine that the limited view through the lens of one discipline is no longer enough For example today medicine must be well versed in nuclear physics because of the tracer techniques and the injury which can result from radiation At the other extreme medicine is certainly a social science and through mental health must be concerned with economic and social questions The answer then is not further fragmentation into increasingly isolated specialties disciplines and departments but the integration of science and scientific knowledge for the enrichment of all branches This integration we feel can be encouraged by providing opportunities for a multiprofessional approach to given topics

Although the fertility of the multidiscipline approach is recognized adequate provision is not made for it by our universities scientific societies and journals And perhaps the presence of other

hindering factors must be admitted Partly semantic in nature, they may also to some degree be psychological Admittedly, it is often times difficult to accept data derived from methods with which one is unfamiliar By making free and informal discussion the central core of our meetings, we hope to achieve an atmosphere which minimizes as much as possible these semantic and emotional barriers

Thus, our conferences are in contrast to the usual scientific gatherings Presentations are designed, not to present neat solutions to tidy problems, but rather to elicit provocative discussion of the difficulties which are being encountered in research and practice We ask that the presentations be relatively brief, and emphasis is placed upon discussion as the heart of the meeting Our hope is that the participants will come prepared not to defend a single point of view but, with open minds, to take full advantage of the meeting as an opportunity to speak with representatives of other disciplines in much the same way as they talk with their colleagues in their own laboratories

During 1953 under the Conference Program conferences will be held on the following topics administrative medicine, adrenal cortex, aging, connective tissues, consciousness, cybernetics, infancy and childhood, liver injury, metabolic interrelations nerve impulse, renal function and shock and circulatory homeostasis

When a new conference group is organized, the Chairman, in consultation with the Foundation, selects fifteen scientists to be the nucleus of the group which will hold annual meetings for a period of five years Every effort is made to include representatives from all pertinent disciplines From time to time, however, new members are added by the group to fill gaps in viewpoint or techniques A *small number of guests is invited to attend each meeting*, but, for the purposes of promoting full participation by all members and guests attendance at any meeting is limited to twenty five During a conference's prescribed lifetime we cannot possibly include more than a small fraction of the key investigators in the field, and one of the difficulties in forming a group, such as this one on renal function is that it is necessary to exclude so many investigators we ould like to include

he transactions of these meetings are recorded and published s is done because the Foundation wishes to make current think in a field available to all those working in it, and to those in r fields who are concerned with science for example, government officials administrators, etc Logic is a vital aspect of science, *equally essential is the intuitive or creative aspect* Research

is as creative as the painting of a portrait or the composing of a symphony Although logic is, of course, necessary in order to rearrange, to test, and to validate, research thrives on creativity which has its source in unconscious, nonrational processes Unfortunately, however, in the research reports which are presented to the world in scientific journals, this integral part of scientific endeavor is shriveled by the cold, white light of logic By preserving the informality of our conferences in the published transactions, we hope to portray more accurately what goes on in the minds of scientists and to give a truer picture of the role which creativity plays in scientific research

FRANK FREMONT-SMITH, M D
Medical Director

ION EXCHANGES BETWEEN EXTRACELLULAR AND INTRACELLULAR FLUIDS

INFORMAL DISCUSSION

Pitts I recently heard Dr Alan Gregg relate how he, as a brash young Harvard freshman, on first meeting the philosopher Josiah Royce, asked 'Dr Royce, what is your concept of heaven?" Without a moment's hesitation, Royce replied, 'Heaven is a state of mind in which I would know from moment to moment the exact significance of my every word and deed" I don't pretend to any such sublime state Rather, I should like to present a few of the experimental deeds of two of my colleagues and have you attach to them their proper significance Dr David Axelrod* and Miss Muriel Seip* are largely responsible for the work that I have to present Dr Swan has some related work which he can introduce in the discussion

I should like to pose two questions Do tissues share their buffering powers with the extracellular fluids when the body is invaded by acid or alkali? Secondly, if they do share their buffering powers, how is that sharing effected?

I will admit that I approached the first question with something of a philosophical bias toward the affirmative Cells contain water, and Darrow, Hastings, and others have shown that they share that water with the extracellular fluid when the body is threatened by dehydration Cells contain buffers, organic phosphates, proteins, and bicarbonate It seems logical that they would share their buffering capacity with the extracellular fluid when the body is threatened with an excess of acid or alkali

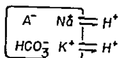
In Figure 1 are pictured some possible mechanisms by which cells and tissues might buffer acid (left) and alkali (right), although I do not wish to imply that these mechanisms exhaust all possibilities Rather I merely wish to suggest that as the hydrogen ion concentration of the extracellular fluid increases, hydrogen ions might be exchanged for cellular sodium or potassium ions The

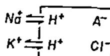
*Department of Physiology Cornell University Medical College

POSSIBLE WAYS BY WHICH TISSUES MIGHT CONTRIBUTE

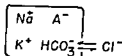
TO THE BUFFERING OF ACID

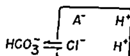
TO THE BUFFERING OF ALKALI

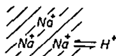


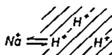


CELLS

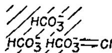








BONE or TENDON



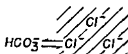


FIGURE 1

converse might be true Extracellular chloride ions might be exchanged for cellular bicarbonate ions In either circumstances, the cells would share their buffering capacity with the extracellular fluids On the other hand, bone might be expected to yield base in exchange for hydrogen ions, because bone contains a considerable excess of sodium over chloride On the other hand, connective tissues in general, and tendons in particular, might participate in an exchange of bicarbonate for chloride, for they contain a relative excess of chloride over base

In the buffering of alkali, the reverse processes might occur, namely, the exchange of hydrogen ions of cells for either sodium or potassium ions of extracellular fluid, or the exchange of chloride of cells for bicarbonate of the extracellular fluid In either instance, the cells would share their buffering capacity with the extracellular fluid Analogous processes might occur in bone and tendon

The problem upon which Dr Axelrod and Miss Seip embarked is the one of tissue buffering of alkali To avoid rapid excretion of administered base in the urine they nephrectomized the dogs at the start of the experiment and maintained them under pentobarbital

anesthesia for the 8- to 10 hour period of observation Nephrectomy also permitted serial observations of volumes of distribution after

control blood sample was drawn from the femoral artery A second control sample was drawn one hour later Data of a typical experiment are presented in Table I The inulin volumes of distribution were observed to be 3320 and 3400 ml in these two control periods The plasma volume, measured by T-1824 during the first period, was 870 milliliters From these data, plasma protein concentrations, plasma ion concentrations, and assumed Donnan factors, it is possible to calculate the quantities of each ion species in the inulin space, a volume designated as the readily diffusible extracellular space Within reasonable limits, the two control series of observations are in agreement

Two hundred and ninety-eight millimols of sodium bicarbonate were infused intravenously in hypertonic solution (6 per cent) Another hour was allowed to elapse to permit distribution Thereafter, three blood samples were drawn at hourly intervals Because the bicarbonate solution was hypertonic, water was withdrawn from cells to expand the extracellular fluid and the volume of distribution of inulin increased accordingly Plasma volume was measured once during the second postinfusion period and was found to have increased to 980 milliliters Repetition of the calculations of ion contents of the readily diffusible extracellular space showed that sodium increased from 491 to 799 mEq, and bicarbonate increased from 91 to 288 mEq, findings which were to be expected qualitatively at least Little change in the potassium content of the inulin space was observed, but the chloride and radiobromide contents increased This finding was unexpected, for no chloride or radiobromide was contained in the infusion

The data from this experiment are summarized in Table II In the first horizontal column are given the mean values for the quantities of sodium, potassium chloride, bicarbonate, and radiobromide found in the inulin space in the two control periods In the second column are given the mean values observed during the three periods following the infusion of bicarbonate In the third column are given the difference values, which may be compared with the quantities of ions administered shown in the next lower column The gross alterations in ion content of the inulin space are given in the

TABLE I

Elapsed Time	Insulin Space	Plasma Volume	Concentration in Plasma Water					Quantity in Readily Diffusible Extracellular space				
			Na^+	K^+	Cl^-	HCO_3^-	Br^{82-}	Na^+	K^+	Cl^-	HCO_3^-	Br^{82-}
mins	*ml of water*		*milliequivalents per liter of water*				*Cts/ml /min*	*milliequivalents*				*Cts·10⁻³ /min*
120	3320	870	151	4 09	117	26 2	1938	483	13 1	404	90 2	6669
175	3400	870	153	4 45	117	26 1	1927	500	14 5	412	92 2	6785
								491	13 8	408	91 2	6727
218 — End of Infusion of 298 Millimols of $NaHCO_3$												
280	4700	980	176	2 95	101	59 9	1673	792	13 3	494	293	8167
340	4750	980	176	2 69	100	58 1	1658	804	12 3	495	287	8184
400	4750	980	176	2 67	103	57 5	1648	802	12 2	506	284	8137
								799	12 9	498	288	8166

Dog 3, 19 0 Kgm

TABLE II

DOG 3 19 0 Kgm	Quantity in Readily Diffusible Extracellular Space				
	Na^+	K^+	Cl^-	HCO_3^-	Br^{82-}
	milliequivalents				*Cts·10^{-3}/min*
Control	491	13 8	408	91	6727
After Bicarbonate	799	12 9	498	288	8166
Δ	+308	– 0 9	+ 90	+197	+1439
Given	298	0	0	298	0
Gross Unaccounted in Readily Diffusible Extracellular Space	+ 10	– 0 9	+ 90	–101	+1439

last column The significant findings may be summarized as follows. All of the sodium of the administered sodium bicarbonate can be accounted for, with relatively minor error, in the inulin space However, some of the administered bicarbonate (–101 mEq) disappeared from the inulin space and was replaced by a roughly equivalent quantity of chloride (+90 mEq) The radiobromide content of the inulin space also increased in rough proportion to the increase in chloride This merely confirms the reality of the chloride shift

Darrow When was the bromide given?

Pitts The bromide was given at the time the inulin was given, two hours or more before the experiment was started No substances other than anesthetic agents were administered to the dog before the kidneys were taken out

Darrow Presumably, there was equilibrium with respect to bromide which had diffused as much as it would?

Pitts Yes, and equilibrium of bromide with respect to chloride These shifts of bicarbonate and chloride between inulin and noninulin spaces are exactly those which are known to occur between plasma and red cells under similar conditions To what extent can the plasma and red cell shifts explain these discrepancies? In Table III are summarized the experimentally determined corrections In each experiment, whole blood, as well as plasma, was analyzed for

each ionic constituent Since the hematocrit was known, the quantity of each ion species in the total circulating red cell mass could be calculated The change observed, in consequence of the infusion of sodium bicarbonate is given in the second horizontal column *It is evident that only a minor fraction of the bicarbonate lost from,* and the chloride gained in, the inulin space can be accounted for by plasma and red cell shifts

TABLE III

DOG 3 19 0 kgm	Unaccounted Quantity in Readily Diffusible Extracellular Space				
	Na^+	K^+	Cl	HCO_3	Br^{82}
	milliequivalents				*Cts·10⁻³/min*
Gross Unaccounted in Readily Diffusible Extracellular Space	+10	—0 9	+90	—101	+1439
Transferred from Circulating Red Blood Cell Volume	+ 3 4	+2 2	— 7 4	+ 10 4	— 183
Net Unaccounted in Readily Diffusible Extracellular Space	+13 4	+1 3	+82 6	— 90 6	+1284

Our *thesis* is simply that when a dose of sodium bicarbonate is given to an animal, the sodium is distributed in the inulin space The bicarbonate remains in part in the inulin space, but in part disappears from that space As bicarbonate disappears, there appears from some space outside the inulin volume of distribution an essentially equivalent quantity of chloride In Table IV are summarized the data of six experiments which were similar to the one just presented except that the dose of sodium bicarbonate was *varied over a range of 178 to 560 milliequivalents The net quantities of sodium, potassium chloride and bicarbonate* unaccounted for in the inulin space, after correction for red cell shifts, are shown in the fourth to seventh columns Sodium was adequately accounted for in experiments 3 through 6, i e with an error of less than five per cent Only in experiments 2 and 6 did appreciable transfers of potassium occur However in all experiments bicarbonate was lost from the inulin space and an essentially equivalent quantity of

given The magnitude of the
less proportional to the dose
is proportionality is shown in

TABLE IV

Dog Number	Weight	Dose $NaHCO_3$	Net Quantity Unaccounted in Readily Diffusible Extracellular Space				$+\Delta Cl$ Extracellular Dose $NaHCO_3$
			Na^+	K^+	Cl^-	HCO_3	
	Kgm	mEq	milliequivalents				percent
1	13.0	178	+24.0	+ 1.4	+ 75.2	− 61.4	12.3%
2	15.0	238	+17.4	+ 4.0	+ 81.9	− 76.6	34.4%
3	19.0	298	+13.4	+ 1.3	+ 82.6	− 90.6	27.7%
4	22.0	357	+17.4	− 1.9	+115.4	−123.6	32.3%
5	24.1	357	+ 6.0	− 0.3	+123.5	−116.6	31.6%
6	20.2	560	+10.1	+10.8	+209	−210	37.3%

the last column of Table IV in which the ratio of the chloride transferred to the dose of sodium bicarbonate given is expressed in percentage terms It can be seen that 27 to 42 per cent of the administered dose of sodium bicarbonate was buffered by a shift of chloride into the inulin space

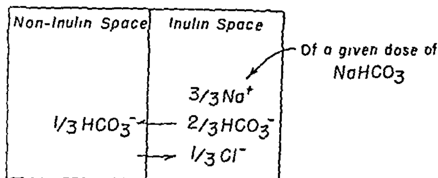


FIGURE 2 Alkali buffering

Figure 2 summarizes our view of the buffering of alkali Of a given dose of sodium bicarbonate three thirds of the sodium ions remain in the inulin space Approximately two thirds of the bicarbonate ions remain in the inulin space but one third disappear A quantity of chloride ions equivalent to about one third of the administered bicarbonate enters the inulin space from some larger space not available to inulin It seems to me that the noninulin space is contributing to the buffering capacity of the inulin space But what does this mean? Are cells contributing their buffers or are bone and connective tissues contributing theirs?

Wallace What was the serum pH of these animals? Did it go up?

Pitts Yes

Berliner How much change takes place in the same period of time in an animal nephrectomized but given no electrolyte?

Pitts In two control experiments there was practically no change In a third control experiment the animal developed a progressive acidosis and changes in pH were observed

Berliner And the inulin space?

Pitts On the whole the inulin space remained relatively" constant

Mudge Were all these infusions hypertonic?

Pitts Yes We wanted to infuse as much bicarbonate with as little water as possible

Darrow How much was the plasma sodium concentration raised?

Pitts From about 156 to 178 in the one experiment in which 298 mEq were given Of course, that varies with the dose of bicarbonate and would differ for the 178 mEq dose and for the 560

Clarke Was the bromide present only in tracer quantities?

Pitts Yes Its chemical concentration was infinitesimal

Thorn Have you tested the distribution of bromide when an isotonic chloride solution was infused?

Pitts To know total body chloride would be helpful We started off with radiobromide with the idea that it could be used as a measure of total body chloride, but unfortunately we did not observe proportional distribution of chloride and bromide between red cells and plasma Therefore, we have not, in this series of experiments, made any special point of total body chloride On the other hand, Dr Swan using ordinary bromide, has observed rather good agreement of bromide and chloride in red cells and plasma Hence, he has used it as a measure of total body chloride

Steinbach This looks like an excellent preparation for observing, *in vivo*, the sort of electrolyte shifts that Mensch has been studying I was wondering whether you had tried any metabolic inhibitors?

Pitts No, we haven t I think the first problem is to find where this exchange occurs Where would the most logical place be to look for it?

Wallace Associated with collagen?

Pitts And what would the best source of collagen be? Not tendon, I think because there isn t very much in the body What mass of collagen is the most logical to use?

Wallace The connective tissue of the body constitutes about 10 per cent of body weight Tendon and perirenal connective tissue [illegible] time ago an ionic composition [illegible] There is a little more chloride [illegible] ltrafiltrate relationship

Darrow About 8 per cent

Wallace Yes Some people in the leather industry, studying collagen have produced evidence that seems to indicate that at least some of the collagen appears to behave like a cation and could be associated with chloride

Pitts Hastings said that the pK of collagen was 4 85 but that it did not bind base at body fluid pH

Wallace I thought he conceived it to be insoluble protein at its iso electric point There are other data that do not agree with that (1)

Darrow Muntwyler's data do not show as great an excess of chloride over sodium in tendons

Wallace The data we obtained in the past year are more like Hastings' findings I asked about the pH change because I was wondering if collagen could be the buffer

Pitts The observed increase in pH would account for some of the loss of bicarbonate, how much I do not know However, chlorides give the best figures, and usually the deficit of bicarbonate exceeds the gain of chloride This discrepancy is no doubt in part due to increased pH

Darrow Did you measure the buffering?

Pitts No

Wallace Respiratory exchange?

Pitts We did not measure respiratory exchange But to return to my question, would the most logical place to look for collagen in the greatest mass, and in the most readily available form, be the skin?

Darrow I cannot give the exact figures, but certain calculations show that a large proportion of the chloride not available to the inulin space lies in skin and connective tissues, and not within the cells This conclusion is based on subtracting the known composition of certain tissues from total body contents This leaves a remainder containing one third to one-half of body chloride and no bulky cellular tissues The contents of this remainder include skin, connective tissues, intestines, and heart The contents of water, sodium chloride, and potassium indicate that a large part of this fluid has a composition resembling extracellular fluid The basis for this statement is supplied by Table V *

The proportionate weights of the various tissues were multiplied by the contents and subtracted from total body contents, as indicated by Widdowson and McCance in analyses of whole bodies

Berliner How about the space into which inulin *does not* diffuse in the time usually allowed for equilibration, but into which it will go if enough time elapses?

Wallace That is the connective tissue space

Pitts Will somebody define for me what is meant by connective tissue space?

*This Table was not presented at the meeting

TABLE V

Composition of One Kg. of Tissue Estimated from Various Sources*

Tissue	Amount Gm	N Gm	Fat Free Solids Gm	H_2O Gm	Cl mEq	Na mEq	K mEq
				Adult			
Muscle	350	12.8	78	292	10.1	13.6	34.3
Brain	22	0.4	3	18	0.9	1.2	1.9
Liver	35	1.0	8	25	1.0	1.2	2.6
Skeleton	170	4.2	95	63	5.2	36.0	4.8
Subtotal	607	18.4	184	398	17.5	52.0	43.6
Total	1000	23.0	204	560	29.7	74.0	56.0
Remainder	393	4.6	20	162	12.2	22.0	12.4
				Infant			
Muscle	210	5.2	41	165	5.9	7.7	16.8
Brain	120	1.7	15	101	5.2	6.6	10.4
Liver	40	1.0	10	29	1.2	1.5	3.2
Skeleton	150	5.2	46	92	7.0	22.0	4.9
Subtotal	520	13.1	112	387	19.3	37.8	35.3
Total	1000	20.1	157	765	50.6	75.6	42.0
Remainder	480	7.0	45	378	31.3	37.8	6.7
			Probable Composition of R B C per Kg Tissue				
	35	1.5	10	24	1.7	0.6	3.2

*Muscle from *J Clin. Investigation* 28, 492 (1949) and autopsy of newborn Brain and liver from cats and kittens, *J Biol. Chem* 123, 295 (1938) Whole bodies from ... analyses of whole ... of an adult by ... msidered to have

Berliner I don't want to try Dr Cotlove (2) has been working with muscle as free of connective tissue as possible Of course, it still contains a good deal of interstitial connective tissue He finds that with infusion of inulin at a constant rate there is a fairly rapid accumulation up to a point where the inulin space in the muscle is about 80 per cent of the chloride space After that, the expans of the inulin space in muscle is very slow, at 15 hours it is of

order of 95 per cent of the chloride space The behavior of the sucrose space in muscle is quite similar

Wallace Of course there is not enough expansion of inulin space to account for this

Berliner No, but there are two other considerations First, there is an expansion of the inulin space, which is of course to be expected But the second question is whether the bicarbonate and chloride can exchange between the inulin space and that part of the extra cellular space which is not included within the inulin space

Pitts But why does not sodium exchange in that same space?

Luetscher Water shifts into the inulin space when hypertonic solution is given In that way, sodium equilibrates without any visible loss, even though there may be a fraction of extracellular fluid outside the inulin space But there would still be a difference in bicarbonate to chloride ratio, caused by the injection, which would equilibrate with any extracellular fluid outside the inulin space When viewed from the standpoint of the inulin space, this would result in a loss of bicarbonate, a gain in chloride, and no loss of sodium, as you observed

Pitts Dr Swan, would you speak about your experiments and throw a little additional light on what the problem may eventually turn out to be?

Swan The manner in which the nephrectomized dog neutralizes a large amount of infused acid has been studied by the technique just described by Dr Pitts with the following modifications Total body chloride was determined by infusing 25 mEq of sodium bromide as a 5 per cent solution and allowing two hours for equilibration Plasma and whole blood concentrations of bromide were determined chemically by the method of Friedman (3) Following two control periods, 0 3 normal hydrochloric acid was infused at the rate of 5 ml per minute for a total of 206 mM, or 700 ml, this concentration of acid and rate of administration having been found by Axelrod to be tolerated by the dog Forty five minutes after the end of the infusion of acid a sample of blood was obtained followed by two more intervals of one hour Data derived from one of these experiments are shown in Table VI The averages of the two control periods before the infusion of acid, and the three periods following the infusion of acid, are shown in Table VII

Following the infusion of acid, the inulin volume of distribution had increased 1 3 liters The amount of sodium in this volume had increased by 158 mEq and the amount of potassium by 38 The chloride in the inulin volume increased 226 and the bicarbonate

TABLE VI

Elapsed Time	Inulin Space	Plasma Volume	Concentration in Plasma Water: Na^+	Concentration in Plasma Water: K^+	Concentration in Plasma Water: Cl^-	Concentration in Plasma Water: HCO_3^-	Total Body Chloride (*from bromide*)	Quantity in Inulin Space: Na^+	Quantity in Inulin Space: K^+	Quantity in Inulin Space: Cl^-	Quantity in Inulin Space: HCO_3^-
mins	*ml of water*		*milliequivalents per liter of water*				*milliequivalents*				
120	3240	900	160	4.20	119	23.4	800	500	13.2	399	79.0
185	3290	790	162	4.47	119	23.6	790	511	14.1	406	81.0
							795	506	13.7	403	80.0
395	End of infusion of 206 Millimols of HCl						1020	644	40.0	623	38.2
425	4500	630	149	9.29	132	6.0	1020	656	50.8	634	35.0
455	4580		149	11.60	132	7.3	1020	691	45.9	629	35.8
550	4560	720	149	10.30	132	7.5	1020	664	45.6	629	36.3

Dog 5, 20.1 Kgm.

TABLE VII

DOG 5 20 4 Kgm	Quantity in Inulin Space				Total Body Chloride (*from bromide*)
	Na+	K+	Cl-	HCO_3-	
	milliequivalents				
Control	506	13 7	403	80 0	795
After HCl	664	46 6	629	36 3	1020
Δ	+158	+31 9	+226	—43 7	+ 225
Given	0	0	206	0	206
Gross Unaccounted in Inulin Space	+158	+31 9	+ 20	—43 7	+ 19

TABLE VIII

Summary of Acid Base Balance

		Exp 5	Exp 6
Na+	Transferred to Inulin Space	158 mEq	139 mEq
K+	Transferred to Inulin Space	31 9 mEq	22 7 mEq
B+	Transferred to Inulin Space	189 9 mEq	161 7 mEq
B+	From HCO_3— in Inulin Space	43 7 mEq	61 mEq
B+	Total in Inulin Space for Neutralizing Acid	232 6 mEq	222 7 mEq
Cl−	Given as HCl	206 mEq	206 mEq
ΔCl−	In Inulin Space	226 mEq	209 mEq
ΔCl−	Total Body Store (*from bromide*)	225 mEq	270 mEq

decreased 43 milliequivalents Total body chloride, as measured by bromide dilution increased *by 225 milliequivalents*

These data, and the data from a similar experiment, are summarized in Table VIII The amount of chloride given as hydrochloric acid is in fair agreement with the observed increase in chloride in the inulin volume of distribution and, in experiment number 5, with the increase in total body chloride These changes are graphically represented in Figure 3 The situation with regard to chloride, prior to the infusion of acid, was as follows Of the

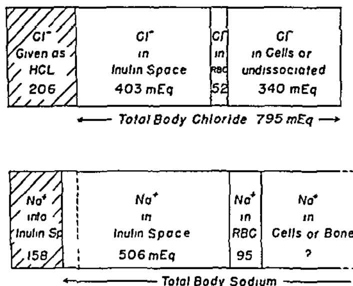


FIGURE 3 Acid buffering Shaded areas *represent increments in sodium and* chloride in the inulin volume of distribution following infusion of hydrochloric acid The unshaded area to the left of the dotted line represents 43 mEq of base contributed by bicarbonate in the inulin volume for the neutralization *of the infused* acid

total chloride of 795 mEq in the body, 403 mEq were in the inulin volume and 52 mEq in the erythrocytes, leaving 340 mEq as the remainder The 206 mEq of chloride *given as hydrochloric acid,* apparently remained within the inulin volume of distribution With regard to sodium, 506 mEq were present in the inulin volume, and 95 mEq in the erythrocytes, prior to the infusion of acid Forty three mEq of the hydrochloric acid were neutralized by base derived *from bicarbonate already present in the inulin volume* The remainder was neutralized largely by sodium which entered the inulin volume from elsewhere *Approximately one-quarter to one-*third of the infused hydrochloric acid was neutralized by base derived from bicarbonate in the inulin volume of distribution *A much larger amount of the infused acid* approximately two-thirds to three-quarters, was neutralized by base entering the inulin volume from elsewhere

Darrow How many mEq of sodium are coming from the non-inulin space?

Swan One hundred and fifty-eight milliequivalents

Darrow One hundred and fifty eight is about 7 mEq per Kg of body weight, isn't it? My estimations indicate that there are about 7 mEq of intracellular sodium per Kg of body weight

Swan Yes, about 8 mEq in this 20 Kg dog, 10 mEq per Kg of hydrochloric acid were infused Following the acid infusion, the amount of sodium in the erythrocytes did not change The amount of chloride in the erythrocytes increased slightly

Thorn Is that the sodium concentration in the red cells?

Swan No, it is the total amount of sodium in the red cells

Selkurt I don't know the immediate relevance of this question but it appeared to me that the plasma volume stayed the same with the acid infusion, but increased with the bicarbonate Is that a consistent finding?

Swan The plasma volume consistently decreased following infusion of acid

Selkurt Although the total extracellular volume went up in both cases?

Swan Yes

Pitts It went up in part because of the volume of fluid infused

Swan Yes 700 ml were infused

Visscher That means that there is a shrinkage of cells somewhere in the body, assuming that the inulin space measures extracellular space How much of the increase in sodium in the one case and chloride in the other could be accounted for simply on the basis of shrinkage of cells that have to maintain constant, or equal osmotic pressure with the external environment for those cells? Have you made that calculation?

Pitts Are you assuming that there is chloride or sodium in those cells?

Visscher I am assuming that there would have to be sodium or chloride containing cells let us say, connective tissue cells in the ligaments which might be involved

Pitts Wouldn't the answer to your question depend on how much sodium and chloride you assume to be in those cells?

Visscher That's right

Pitts So you can come out with the proper answer if you select the proper concentration

Visscher Yes, but my point is really that you haven't made one type of calculation which I would like to see, namely, what the changes in cell water would have to be [illegible] account for the inulin space changes Als[illegible] 'd like [illegible] here the cell water came from

Wallace Dr Visscher, where are chloride-containing cells to be found other than in the testis and red blood cells?

Visscher In the mucosa of the intestinal tract

Darrow Not quantitatively impressive with respect to total body weight

Mudge Does not liver contain intracellular chloride?

Darrow Not much, relatively The liver constitutes only about four per cent of the body weight

Wallace The amount of intracellular chloride in the liver is infinitesimal in an experiment like this

Darrow I can t account for large amounts of extracellular chloride in any body cells that I know of, paying attention to the proportionate weight of the tissue in relation to total body weight

Mudge I should like to ask if any studies have been done on the equilibration of isotopic chloride with chloride of the whole body in mammals Does *all* chloride exchange? Is some "nonexchangeable"? Or are there slow components in some tissues? There is, of course, a large reservoir of body sodium which does not readily exchange In Figure 3 Dr Swan used the term "undissociated chloride" I wondered just what was meant by it

Ussing I don't know, but I presume it would mix entirely with it

Mudge There is a big reservoir of body sodium which does not mix

Ussing Yes, but that is probably simply a steric hindrance by the crystal lattice

Swan Infused bromide equilibrates with the chloride in erythrocytes within two hours The dilution of bromide gives a value for chloride per kg of body weight which agrees with that reported in the literature (1) The infusion of hydrochloric acid results in an increase in plasma chloride concentration of 10 to 15 mEq per liter It appears that chloride outside the inulin volume of distribution does not increase in concentration as the plasma concentration of chloride increases

Mudge I don't think that means "undissociated," does it?

Swan No that is merely one possibility Another possibility is that the chloride which is not in the inulin volume is intracellular

Elkinton There is a big difference between the two isotopes that have been used, that is in respect to their half lives The one with a very short life is, I think, Cl^{38} As far as I know, the only human experiment with this isotope was in a patient of Francis Moore's, where I think the chloride space accounted for 18 per cent of the body weight (5) We used this isotope in dogs and got about 25 per

cent, as compared with 28 per cent for radioactive sodium (6) I think that George Burch (7), however, has used Cl^{36}, the very long-life isotope It is easier to work with, for obvious reasons, but it is very hard to get I believe Burch found something like 30 to 34 per cent Does anybody know whether he found differences in the rate of diffusion of that isotope which would suggest, perhaps, two different compartments?

Pitts Weil did experiments of that sort with Cl^{36} He is one of the few who apparently can afford to use it We have some, but I estimate that each experiment would cost about $200

Wallace James Gamble, Jr, who is now at Brookhaven National Laboratory, has some excellent data on chloride distribution on the dog * Using Cl^{38} and Cl^{36}, he found that chloride reaches a constant volume of distribution in about an hour, and remains constant for as long as 24 hours

Mudge Is this by analysis of individual tissues?

Wallace By analysis of the kinetics of distribution

Elkinton What distribution is found?

Wallace In dogs he found a volume of distribution of about 25 per cent. His inulin spaces are about the same as others we have found

Elkinton And you interpret the difference as collagen?

Wallace I don't see where it could be except in connective tissue, as the tissues in the body that have enough chloride in them to account for it are limited to a few testis, red cells, and possibly liver

Darrow The white blood cell is supposed to have intracellular chloride

Steinbach If all the past literature were forgotten, and these were the only experiments on the subject, wouldn't they be used to prove the existence of sodium and chloride spread generally through tissue cells?

Pitts I suppose so

Steinbach Why don't you do it, then?

Pitts With respect to buffering, there are a number of possibilities, and I don t believe our experiments to date are conclusive as to which is correct In Figure 2 were shown only those possibilities for which some evidence existed For the buffering of alkali, our data suggest the possibility of the exchange of bicarbonate for

*Gamble J L Jr and Robertson J S The volume of distribution of radioactive chloride in dogs comparison with sodium bromide and inulin spaces (Unpublished observations)

chloride in cells or in some supporting structure such as connective tissue or tendon However from Darrow's work one would tend to believe the exchange of sodium for hydrogen more probable On the other hand Dr Swan's data on the buffering of acid suggest that sodium is exchanged for hydrogen

Steinbach In every case where a single animal cell has been analyzed sodium and chloride are found internally All the claims for chloride free compartments in the body are based on indirect measurements

Wallace Isn't there some rationale for considering chloride extracellular Dr Steinbach? The amount of chloride in most of the body tissues is such that if any of it were intracellular practically no extracellular space would be left It would be hard to reconcile with the morphology as we know it Are there chemical gradients for chloride?

Steinbach No There are certainly differential distributions of chloride I am not saying that it is evenly distributed according to chemical gradients If electrochemical gradients were considered and if intracellular chloride were assumed I doubt that it would involve much more than a small percentage change in extracellular space calculated as chloride space and yet there would be a sufficient amount to account for all of Dr Pitts data It seems to me that the data shown are good enough so that if they don't agree with the calculated spaces it is better to assume that the spaces are wrong than throw out the theory

Mudge In connection with the direct measurements of isolated tissues I should like to ask Dr Steinbach whether that holds true for mammalian skeletal muscle which is after all the cause of the argument

Steinbach That argument can't be settled here because we have no single cell measurements Isolated frog muscle fibers have been analyzed for chloride In all the cases where one can collect single cells and know what the distribution space is intracellular sodium and chloride are found in about the distribution that Dr Ussing would predict on the basis of his studies I tend to doubt the calculations on mammalian tissue which assume no intracellular chloride simply because electrochemical gradients are probably such that there is very little intracellular chloride but enough to account for a buffering action such as Dr Pitts requires The calculations would be very interesting perhaps Dr Pitts has made them How much chloride or sodium has to be put into muscle cells brain cells

etc in order to account for the results? I don't think it would be much

Pitts You made some rough calculations on that didn't you Dr Swan?

Swan In the case of experiments in which hydrochloric acid was infused if all of the sodium entering the inulin volume to neutralize hydrochloric acid were assumed to come from muscle cells intra cellular bicarbonate concentration would be reduced to practically zero

Wallace I am sure that appreciable amounts of sodium can be mobilized from bone within six hours Bone sodium is very labile in acidified animals

Darrow Does it come out with calcium?

Wallace If it does it doesn't affect the argument However sodium and calcium do seem to be removed from bone at different rates under conditions of acidosis

Thorn Most of us would agree that sodium and chloride enter body cells However in a major operation with all of the stresses involved particularly in an animal under anesthesia the question arises whether there has not been an appreciable shift of sodium and chloride in the body cells before the other aspects of the experiment have been considered This sodium and chloride might be available as a source of increasing the inulin space

Pitts These animals were acutely nephrectomized

Thorn Yes but since an acute nephrectomy is a major operation it may greatly influence the sodium and chloride content of body cells

Steinbach During and following operative procedures the change is toward building up the store of sodium and chloride in the cell

Thorn Yes

Visscher It could be tested by measuring the inulin space and the plasma chloride before and after surgery Did they change?

Pitts Although we didn't do the experiment we have the data on which to argue that point We found an inulin space of around 20 per cent of body weight the same value as in a normal dog Furthermore the plasma chloride concentrations at the start of the experiment were certainly within the normal range I think that this question of sodium and chloride going into the cells because the animal is given pentobarbital anesthesia or nephrectomized is stretching the point a bit

Thorn I think that a bilateral nephrectomy is a sufficiently severe procedure to cause a shift of sodium and chloride in the body cells

Pitts The operation puts sodium and chloride into the cells?

Thorn A decrease in plasma sodium concentration is often observed in the postoperative surgical picture Accompanied by a retention of sodium, this suggests either water loss from the cells to the extracellular fluid, or sodium and chloride transfer to the cell, or both It would be necessary to measure extracellular fluid volume, and calculate the total sodium chloride content of the extracellular fluid under these circumstances before one could be certain that appreciable sodium chloride shift had occurred However, the clinical fact of marked sodium retention, with a decrease in serum sodium level, certainly makes one entertain the possibility of loss of sodium to cells (8)

Visscher Dr Pitts, you are confronted with the necessity for answering certain dogmatic opinions one way or the other

Pitts I should say that all calculations made on the assumption that chloride is extracellular, and that all chloride present in the body is in diffusion equilibrium with a protein-free filtrate of the plasma are highly suspect

Steinbach That is a bold statement

[illegible]

large lump in a muscle

Pitts I am not saying this is in terms of muscle

Darrow There are only 7 or 8 mEq in all the muscles of 1 Kg of dog I don't think it would all come from the muscle, since a large part of muscle chloride must remain extracellular

Ussing Why is it so important to come to the conclusion that chloride is never present in cells?

Swan There isn't much intracellular chloride in muscle, and muscle comprises a very large fraction of the cell mass of the body

Ussing After all, the total amount in organs is so large that even a low concentration would help, wouldn't it?

Swan Could it have made a significant contribution to the 4 mEq of chloride per Kg of body weight which entered the inulin volume following the infusion of bicarbonate?

Darrow The total chloride in the muscle, as I calculate it, cannot be over 7 or 8 mEq per Kg of body weight, and it would be hard to conceive of 4 mEq of chloride as being in the muscle cells If 1 mEq per Kg of body weight comes from the cells, most of the intracellular chloride has to come from other cells It isn't a ques-

tion of whether there is chloride in the cells, but of how much it is My guess is that the total is on the order of 5 mEq per Kg of body weight That is just a guess, based on what I know of tissue analysis

Pitts That is piecemeal?

Darrow No, that is total intracellular chloride in 1 Kg of body weight This assumes intracellular chloride in liver and other tissues according to their weights, and assigns chloride in a fairly liberal proportion of the total chloride content of the various tissues as intracellular I can't make up my mind that there are more than about 4 to 5 mEq of chloride in the cells of 1 Kg of tissues having the composition of the body as a whole

Pitts Your assumption is based on the chloride space, Dr Darrow, which is some 30 per cent of body weight There is the hitch When 30 per cent of body weight is considered to be extra cellular space, little chloride is left for the cell

Darrow No, no!

Pitts If 15 to 20 per cent of body weight is considered to be extracellular there is plenty of chloride for the cells

Darrow That is much more than is known to be in cells

Pitts In muscle? After all, the total body chloride is the same under either circumstance

Darrow In any of the tissues The other tissues don't weigh enough to account for 40 per cent of the total body chloride in cells

Pitts But there is more in some of the tissues

Darrow What tissues can contain large amounts of chloride in cells? Liver is 4 per cent of body weight, the heart is a negligible percentage of total weight, the brain is 2 per cent of total weight Where are large amounts of intracellular chloride? One can assume that half the chloride in the liver is intracellular It still doesn't give very much That is what bothers me I do believe there is considerable chloride in the cells but I can't account for 40 per cent of the chloride being there

Elkinton Twenty per cent of the body is connective tissue, which is very high in chloride and sodium

Darrow Yes, but I do not consider connective tissue chloride as truly intracellular

Elkinton Dr Wallace, didn't you find that sodium moved, just as chloride did, into the connective tissue phase that inulin didn't enter?

Wallace That's right As serum chloride falls, tendon chloride falls Muntwyler demonstrated that Similarly, as serum sodium

changes, the concentration of sodium in connective tissue seems to run parallel

Elkinton There is a major difficulty in using the connective tissue, noninulin-containing phase to explain this shift which Dr Pitts has shown he apparently didn't find the sodium going into that phase He accounted for all of his sodium in the inulin space I am surprised at that because in the human subjects to whom we have been giving hypertonic sodium bicarbonate, we have found *that the sodium went very rapidly into* a space amounting to 20 or 21 per cent of body weight much more than the inulin space We interpreted it as probably going into the connective tissue phase (9) This was before the slow diffusion, perhaps into muscle cells

Steinbach What is the inulin space and the so called chloride space for mammalian muscle? I can t remember

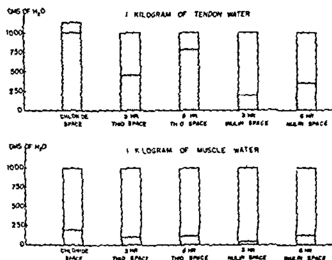


FIGURE 4 A comparison of measures of extracellular tissue fluid The distribution of inulin and thiosulfate at three and six hours in tendon and muscle water as compared with chloride From Nichols G *et al* Unpublished data

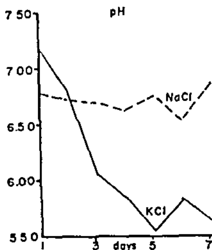
Wallace Figure 4 shows measurements made directly on inulin and chloride in tendon and muscle The situation in skin is similar to that in tendon *It can be seen that* when the chloride space in tendon is calculated from serum data it comes out to more than 100 per cent of the water of tendon The thiosulfate and inulin spaces of tendon are much smaller than the chloride spaces In the

lower part of the Figure are given the same measurements for muscle In six hours neither thiosulfate nor inulin seems to diffuse into as much water as chloride does The inulin space in muscle is much smaller than the chloride space but tends to increase with time Dr Cotlove (2) finds the same thing Skin seems to behave similarly There is a lot of skin in the body and of course muscle liver and all the rest of the organs contain connective tissue

Pitts I suggest that we continue with the discussion of ion exchanges between cells and their fluid environments by asking Dr Darrow to speak of his experiments

Darrow Essentially our experiments consisted of the production of alkalosis with potassium deficiency in animals by feeding a diet low in sodium potassium and chloride while giving them a solution to drink containing sodium and chloride in a ratio of about 1 5 to 1 and in addition giving desoxycorticosterone 2 mg a day for about three weeks Thereafter the animals were kept on the same diet with distilled water to drink while the desoxycorticosterone was withdrawn After three days urine was collected for control and the animals were then treated in two ways one group received 3 mM of potassium chloride twice a day intraperitoneally and the other group received 3 mM of sodium chloride twice a day intra peritoneally The excretion of electrolyte in the urine and the changes in muscle composition and serum concentrations were followed The purpose of the experiments was to observe the effects of sodium chloride and potassium chloride in alkalosis The rats all had moderate metabolic alkalosis at the onset of treatment The muscles were deficient in potassium and had an excess of intra cellular sodium

The Figures show the average values of daily urine electrolyte excretion in a group of eight animals Figure 5 shows that the urinary pH of the animals receiving potassium chloride became more acid while in those receiving sodium chloride it remained essentially the same as before treatment In Figure 6 the bicar bonate concentration as would be expected dropped in the urine of the animals receiving potassium chloride but remained essen tially the same in the animals receiving sodium chloride The output of titratable acidity of course increased in the animals receiving potassium chloride but remained essentially the same in the animals receiving sodium chloride as shown in Figure 7 We were not cer tain that the estimations of the ammonia excretion were absolutely satisfactory There was a good deal of variation in them but if anything there was no difference in the ammonia excretion of the



F ... with NaCl and KCl Reprinted, by
p ... Cheek D B., Coville F E. and
I ... alkalosis associated with potassium
c

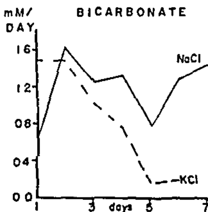


FIGURE 6 Daily urinary bicarbonate excretion. Reprinted by permission, from Cooke R. F *et al.* The extrarenal correction of alkalosis associated wi h potassium deficiency J Clin. *Investigation* 31, "98 (1952)

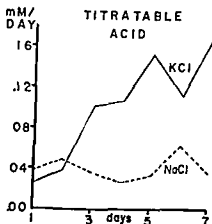


FIGURE 7 Total daily titratable acidity of urines Reprinted, by permission from Cooke R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, *798 (1952)*

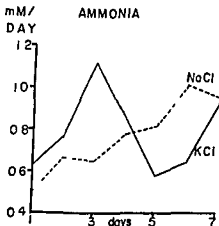


FIGURE 8 Total daily urinary ammonia excretion Reprinted by permission from Cooke R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, *798 (1952)*

two groups of animals, as noted in Figure 8 As Figure 9 shows, the phosphorus excretion was essentially the same in the two groups, although there is evidence of some increased excretion in the sodium chloride groups on the first day, associated with a decrease in the potassium chloride group The meaning of this is not clear

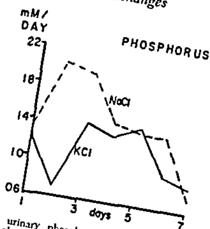


FIGURE 9. Total daily urinary phosphorus excretion. Reprinted by permission from Cooke, R. E., et al. The extrarenal correction of alkalosis associated with potassium deficiency. *J. Clin. Investigation* 31, 798 (1952).

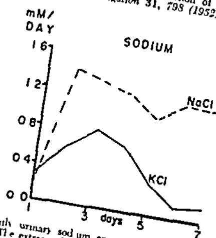


FIGURE 10. Total daily urinary sodium excretion. Reprinted by permission from Cooke, R. E., et al. The extrarenal correction of alkalosis associated with potassium deficiency. *J. Clin. Investigation* 31, 798 (1952).

Figure 10 records the averaged sodium excretions. The animals receiving sodium chloride excreted essentially all the sodium chloride given and the animals receiving potassium chloride started to excrete sodium quite promptly despite the fact that there was none in the diet or injections. Sodium excretion reached a peak in about three days and then gradually decreased in six to seven days so that practically no sodium was being excreted at the end of the period of observation.

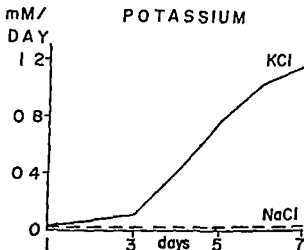


Figure 11 Total daily urinary potassium excretion Reprinted by permission from Cooke R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* 31 798 (1952)

Potassium excretions are shown in Figure 11 As expected the animals receiving sodium chloride excreted almost no potassium while those receiving potassium chloride excreted practically none during the first three to four days despite administration of large amounts of potassium chloride Potassium excretion gradually approached the level of the intake after six or seven days The chloride excretion in the urine in both groups approached that of the intake very quickly as Figure 12 demonstrates

In the animals receiving potassium chloride the serum concentrations and the muscle analyses indicated that there was essentially complete recovery in six to seven days while the animals receiving sodium chloride showed no tendency to recover from the alkalosis or to restore their muscle composition We have the paradox of recovery from alkalosis during administration of potassium chloride while the animals were excreting a more acid urine and no bicarbonate in other words the urine findings cannot explain the recovery from alkalosis unless we also take into account the exchanges at the cell membranes between extracellular and intracellular fluids

Table IX shows the figures for the serum concentrations The first line shows the normal concentration of sodium potassium chloride and bicarbonate The second line shows that the degree of alkalosis was not very great The average serum bicarbonate was

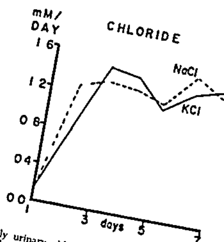


FIGURE 12 Total daily urinary chloride excretion. Reprinted by permission from Cooke, R. E., *et al.* The extrarenal correction of alkalosis associated with potassium deficiency. *J. Clin. Investigation* 31, 795 (1952).

TABLE IX

Average Serum Concentrations in Alkalosis with K Deficiency (mM per l) Treated

Group	No	Days	pH	Na	K	Cl	HCO_3
Normal			7.28	143	5.4	103	21
K deficient	8	0	7.46	143	2.7	93	30
NaCl 6 mM	8	6	7.46	143	2.9	96	30
KCl 6 mM	7	1	7.50	141	3.3	96	26
KCl 6 mM	6	2	7.39	141	4.3	103	23
KCl 6 mM	7	3	7.39	141	4.7	101	23
KCl 6 mM	4	4.5	7.43	141	4.4	101	24
KCl 6 mM	2	6	7.45	141	4.6	105	23

Treated rats received 3 mM per Kg twice daily

Reprinted by permission from Cooke, R.E., *et al.* The extrarenal correction of alkalosis associated with potassium deficiency. *J. Clin. Investigation* 31

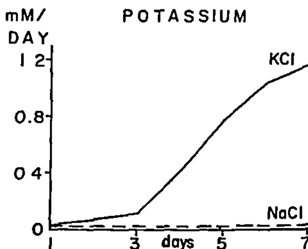


FIGURE 11 Total daily urinary potassium excretion Reprinted by permission, from Cooke R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, *798 (1952)*

Potassium excretions are shown in Figure 11 As expected, the animals receiving sodium chloride excreted almost no potassium, while those receiving potassium chloride excreted practically none during the first three to four days, despite administration of large amounts of potassium chloride Potassium excretion gradually approached the level of the intake after six or seven days The chloride excretion in the urine in both groups approached that of the intake very quickly, as Figure 12 demonstrates

In the animals receiving potassium chloride, the serum concentrations and the muscle analyses indicated that there was essentially complete recovery in six to seven days, while the animals receiving sodium chloride showed no tendency to recover from the alkalosis or to restore their muscle composition We have the paradox of recovery from alkalosis during administration of potassium chloride, while the animals were excreting a more acid urine and no bicarbonate, in other words, the urine findings cannot explain the recovery from alkalosis unless we also take into account the exchanges at the cell membranes between extracellular and intracellular fluids

Table IX shows the figures for the serum concentrations The first line shows the normal concentration of sodium, potassium, chloride and bicarbonate The second line shows that the degree of alkalosis was not very great The average serum bicarbonate was

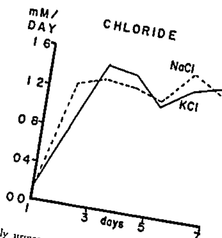


FIGURE 12 Total daily urinary chloride excretion Reprinted, by permission, from Cooke, R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, 798 (1952)

TABLE IX

Average Serum Concentrations in Alkalosis with K Deficiency (mM per L) Treated

Group	No	Days	pH	Na	K	Cl	HCO_3
Normal			7 28	143	5 4	103	21
K deficient	8	0	7 46	143	2 7	93	30
NaCl 6 mM	8	6	7 46	143	2 9	96	30
KCl 6 mM	7	1	7 50	141	3 3	96	26
KCl 6 mM	6	2	7 39	141	4 3	103	23
KCl 6 mM	7	3	7 39	141	4 7	104	23
KCl 6 mM	4	4 5	7 43	141	4 4	101	24
KCl 6 mM	2	6	7 45	141	4 6	105	23

Treated rats received 3 mM per Kg twice daily

Reprinted, by permission, from Cooke R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, 798 (1952)

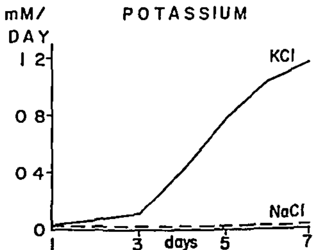


FIGURE 11 Total daily urinary potass um excretion Repr nted by permiss on from Cooke R E *et al* The extrarenal correct on of alkalosis associated with potass um defic ency *J Cl n Investigation* **31**, *798 (1952)*

Potassium excretions are shown in Figure 11 As expected the animals receiving sodium chloride excreted almost no potassium while those receiving potassium chloride excreted practically none during the first three to four days despite administration of large amounts of potassium chloride Potassium excretion gradually approached the level of the intake after six or seven days The chloride excretion in the urine in both groups approached that of the intake very quickly as Figure 12 demonstrates

In the animals receiving potassium chloride the serum concentrations and the muscle analyses indicated that there was essentially complete recovery in six to seven days while the animals receiving sodium chloride showed no tendency to recover from the alkalosis or to restore their muscle composition We have the paradox of recovery from alkalosis during administration of potassium chloride while the animals were excreting a more acid urine and no bicarbonate in other words the urine findings cannot explain the recovery from alkalosis unless we also take into account the exchanges at the cell membranes between extracellular and intracellular fluids

Table IX shows the figures for the serum concentrations The first line shows the normal concentration of sodium potassium chloride and bicarbonate The second line shows that the degree of alkalosis was not very great The average serum bicarbonate was

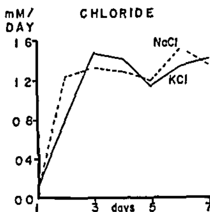


FIGURE 12 Total daily urinary chloride excretion Reprinted by permission from Cooke R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, *798 (1952)*

TABLE IX

Average Serum Concentrations in Alkalosis with K Deficiency (mM per L) Treated

Group	No	Days	pH	Na	K	Cl	HCO_3
Normal			7 28	143	5 4	103	21
K deficient	8	0	7 46	143	2 7	93	30
NaCl 6 mM	8	6	7 46	143	2 9	96	30
KCl 6 mM	7	1	7 50	141	3 3	96	26
KCl 6 mM	6	2	7 39	141	4 3	103	23
KCl 6 mM	7	3	7 39	141	4 7	101	23
KCl 6 mM	4	4 5	7 43	141	4 4	101	24
KCl 6 mM	2	6	7 45	141	4 6	105	23
Treated rats received 3 mM per Kg twice daily							

Reprinted by permission from Cooke R.E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, *799 (1952)*

had been given By the third day, muscle potassium was 45 mEq per 100 Gm of fat free solids By the sixth day, the muscle composition returned practically to normal

Figures 13 and 14 show the cumulative balance between the intakes and urinary outputs The data agree with the tissue analyses The average cumulative balance in the animals receiving sodium chloride is shown first (Figure 13) These values are expressed per Kg of body weight There may be a slight loss of potassium according to the balance data, and there is a slight retention of sodium and chloride, perhaps partly attributable to cumulative errors, but it may also be due to slight expansion of extracellular fluids In any case, the muscle concentrations did not seem to show evidence of retention of chloride and sodium

Figure 14 shows the cumulative balance for the group receiving potassium chloride, and here you see retention of potassium which amounts to almost 20 mM per Kg by the sixth day, and a gradual cumulative loss of sodium as potassium is retained There is a slight retention of chloride These figures may be somewhat erroneous owing to the fact that we did not take into account the skin or stool losses But I think they are reasonably good balances because the intake did not involve the errors that occur in rat feeding experiments in which food is spilled All the electrolyte intake was given subcutaneously so that we think these balances are pretty good We therefore have errors only in urine collection and no significant errors in intake

[illegible] the muscle
[illegible] um
[illegible] um
concentration rises We think that it is the retention of potassium within the cells that accounts for the increase in the titratable acidity Since the potassium was given as potassium chloride the excretion of chloride accounts for most of the excess excretion of anions Figure 16 shows the fall in muscle sodium and the rise in the excretion of sodium I think there can be little doubt that the excreted sodium by the animals receiving potassium chloride comes from the muscle

Figure 17 is a relatively simple diagram of a cell with a nucleus which indicates our view that the potassium is exchanged for sodium during correction of alkalosis The exchange apparently goes on in the ratio of three potassium ions entering the cell for two sodium ions leaving indicating a net balance in which there is a shift of one hydrogen ion out of the cell In other words,

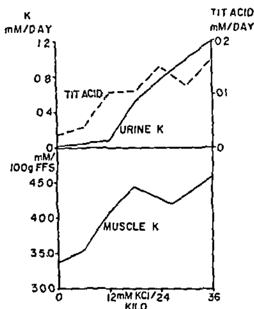


FIGURE 15 Muscle K contents related to urinary titratable acidity and K in rats treated with KCl Reprinted by permission from Cooke R E *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, *798 (1952)*

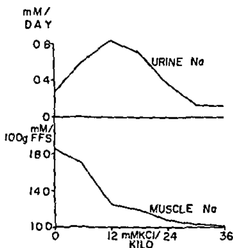


FIGURE 16 Muscle Na contents related to urinary Na excretion in rats treated with KCl Reprinted by perm ss on from Cooke R E *et al.* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* **31**, *798 (1952)*

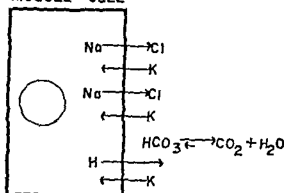


FIGURE 17 Diagram of cation exchange during recovery from alkalosis Reprint by permission, from Cooke R E, *et al* The extrarenal correction of alkal associated with potassium deficiency *J Clin Investigation* 31, 798 (1952)

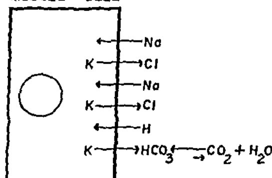


FIGURE 18 Diagram of the membrane exchange during the production of alkalosis Reprinted, by permission, from Cooke, R E, *et al* The extrarenal correction of alkalosis associated with potassium deficiency *J Clin Investigation* 31, 798 (1952)

such an exchange explains the drop in the concentration of bicarbonate in extracellular fluids by the formation of carbon dioxide and water The reverse process must take place

In other words, in the development of potassium deficiency (Figure 18), three potassium ions must be exchanged for two sodium ions that enter the cells, and one of the potassium ions must be exchanged for a hydrogen ion to preserve neutrality Thus, in this type of alkalosis we have a deficit of base within the cells which is greater than the deficit of chloride in the extracellular fluids It is not at all surprising that the recovery from such an extracellular alkalosis should involve the excretion of acid urine This sort of ionic exchange may take place in many other processes

Pitts Dr Darrow may I interrupt for a moment? Were these animals pair fed? Was the food intake constant? Were all the animals equally potassium deficient?

Darrow Roughly, they were, but they were not pair-fed We felt it was not necessary to use pair feeding with diets giving a low intake of anions and cations The load of anions and cations was chiefly obtained from the injections which were commensurate

Pitts It was an acid ash type of diet, was it not, since it contained very little base? The thing that disturbed me when I read your paper, and still does probably because I don't comprehend its significance, is the increased titratable acidity of the urine in the presence of increased excretion of potassium It is very easy to demonstrate in the dog that if one infuses potassium chloride, or gives an oral load of the salt the urine becomes less acid, or frankly alkaline If you infuse large amounts of potassium chloride, the reabsorption of bicarbonate is decreased to less than half its normal value The urine becomes markedly alkaline and large amounts of bicarbonate bound base are lost, which produces an extracellular acidosis I wonder if the acid-ash content of the diet may not have something to do with increased acidity and also with the increased titratable acidity of the urines in these experiments

Berliner I think I might be able to answer that question Drs *Orloff and Kennedy and I were concerned with the same problem* and decided to do what Dr Darrow has been talking about, i e, to see what happens in the animal without kidneys We took similarly prepared potassium-depleted, alkalotic rats nephrectomized them, and then compared the results of infusing sodium chloride and potassium chloride When that was done, the ones that received potassium chloride presented in three to four hours, a plasma

bicarbonate concentration quite significantly lower than the or that were given sodium chloride

Pitts I may say I was certainly in favor of the general idea Dr Darrow However I think that does not necessarily answ the question about the titratable acidity of the urine

Berliner I agree

Darrow We must admit that it would have been better if could have given you the exact data on intakes in the diet b we could not do that and I cannot recall the exact composition the salt mixture in this diet I think it had a rather low acidit However the urines showed little change in phosphorus excretio but did show a difference in excretion of cations Considering tl amounts of potassium chloride or sodium chloride given essential all chloride appeared in the urine in both groups In the rats recei ing sodium chloride all sodium also appeared in the urine Tl rats receiving potassium chloride excreted little potassium until tl third day and the amount of sodium excreted was less than tl potassium retained I should think it was the load of the chloric that accounted for the titratable acidity rather than other dietar ions Nevertheless the experiments are open to the criticism th the dietary loads of the food are not precisely defined The foo intakes were weighed and were about the same

What sort of equilibrium exists between the extracellular flui and the muscle? A few years ago we published Figure 19 in whic we showed the bicarbonate concentration in serum plotted agains muscle potassium (on the left) and sodium (on the right) Ther is evidently a relation between the bicarbonate concentration i serum and the muscle composition with respect to sodium an potassium (10 11 12)

These studies involved rather particular conditions and represen what we called a biological equilibrium or renal adjustment to the deficit of one of the ions sodium chloride or potassium These studies have been extended somewhat in rats that have beer exposed to high tension of carbon dioxide for long periods Under those circumstances a respiratory acidosis develops which is com pensated by very high bicarbonate concentrations as high as 40 mEq per liter but the muscles show no change in composition Therefore I think that the original diagram can be used only to express the relationship in the metabolic acidosis and alkalosis We could have expressed the relationship in general terms if we had related muscle composition to pH rather than to bicarbonate because the points of respiratory acidosis and metabolic acidosis

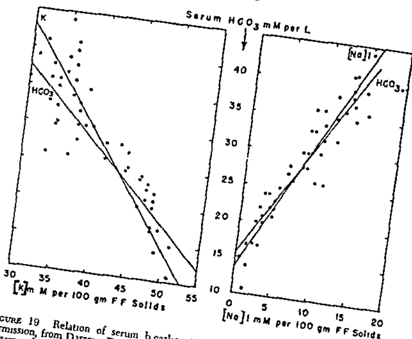


FIGURE 19. Relation of serum bicarbonate to muscle composition. Reprinted by permission, from Darrow, D. C., Schwartz, R., Iannucci, J. F., and Coville, F.: Relation of serum bicarbonate concentration to muscle composition. *J. Clin. Investigation 27*, 198 (1948).

and alkalosis fall along the same pH lines. But be that as it may, it seems to me that this relationship of extracellular pH to cellular composition has to be considered in discussing the equilibrium between extracellular and intracellular fluids.

Now there are so many objections to the Donnan equilibrium that it is likely to be discarded by most people, but Conway has advanced a modified Donnan equilibrium for frogs muscles. His work on frog muscle was essentially as follows. He soaked the frog muscle in varying concentrations of potassium chloride. From the concentrations in the fluid and in the muscle cells he derived certain relationships. These relationships represented a modified Donnan equilibrium when potassium was sufficiently high outside of the cells. The following are the relationships which he showed:

$$\frac{(K)e}{(K)c} = \frac{(Cl)c}{(Cl)e} = \frac{(HCO_3)c}{(HCO_3)e} = \frac{(H)e}{(H)c} = R \; (pHe - pHc) = \log R \qquad [1]$$

What evidence is there for this relationship *in vivo* using the data available in muscle analyses? With increase or decrease in extracellular potassium concentration intracellular potassium seems to change according to the ratio, with appropriate changes in the chloride ratio However, this statement involves some uncertainty as to intracellular chloride concentration And the bicarbonate concentration in the cells cannot be reconciled with the theoretical ratio, even approximately This means that the hydrogen ion ratio is also different The probable potassium ratio demands a concentration of bicarbonate in the cells of something less than 1 mEq per liter of water Conway, of course, thinks that this concentration is probably right, and he defends his view by determining the barium insoluble bicarbonate in muscle, but I have never been quite sure that it is reliable Conway gets figures of the right order of magnitude, by this method I know Dr Wallace wouldn't think very much of it, because he determined the total carbon dioxide of the muscle, and I think his figures come to around 10 to 12 mEq per liter of cell water

Wallace In normal muscle, per Kg of water

Darrow Is that for the muscle water or for the intracellular water?

Wallace Intracellular, corrected to a water basis

Darrow Now if Conway's idea of an extremely low bicarbonate is true, it would require a pH in the cells of around 5 95, which I think is lower than most people believe I don't believe that Dr Wallace would defend the idea that all of the carbon dioxide liberated by acid is derived from bicarbonate, but I think that most of it is If you use his figure I think it would yield a pH somewhat higher 6 1 to 6 2

Wallace It is higher than that 6 9

Darrow I think that is probably a little too high

Wallace One trouble with either the pH we calculated or the one that Conway did is that according to Faurholt (13) no carbamino carbon dioxide can exist at either of these values Conway points this out in his paper and is very careful not to call it carbamino

Darrow Well, I think th[illegible] the pH of the cells prol[illegible] is a little [illegible] mates 6 [illegible] pH I think

EDITOR'S NOTE — Dr [illegible] ference "afterthought [illegible] ies to [illegible]
The discrepancy in Wall[illegible] [illegible]wing Con-
the am[illegible] arbon d[illegible] to [illegible] for by

Wallace I should like to repeat that there must be a very peculiar compound to make carbamino carbon dioxide in appreciable quantity at pH 6 2

Darrow I am getting way over my head What I want to suggest is that the whole idea of using the Donnan equilibrium involves two difficulties (*a*) the sodium concentration within the cells which can be said to be outside a diffusion equilibrium and also I think (*b*) the bicarbonate concentration within the cells Our problem is to develop some theory to explain intracellular sodium and bicarbonate concentrations Whatever theory we develop we have to deal with the facts that are described in the Donnan equilibrium We have to treat the changes in composition of cells in terms of ionic and osmotic forces There must be electric neutrality and osmotic equilibrium Some ions freely diffuse and will respond promptly to changes in reaction or osmotic pressure independent of energy production by cells

TABLE XI

	$\frac{(HCO_3)_c}{(HCO_3)_e}$	pH_c	pH_e	B_c+	B_e+
Normal Animal	0 40	6 98	7 38	204	153
Potassium Deficient Animal	0 16	6 63	7 44	188	155

Average values for calculated bicarbonate ratios between muscle cell water and serum water calculated pH of cellular water (pH_c) serum pH (pH_e) total base concentration in cellular water (B_c+) and extracellular total base (B_e+) in normal and potassium deficient rats

From Schwartz R and Wallace W M Unpublished data

Wallace Table XI summarizes some data we obtained on rats made potassium deficient very much as Dr Darrow has just described but without the administration of desoxycorticosterone The normal values are shown at the top of the Table The calculated distribution ratio for bicarbonate is 0 4 This is calculated assuming that all of the carbon dioxide is bicarbonate The extracellular pH in the normal animals is 7 38 The total base concentration in intracellular and extracellular water is shown The calculations are made on the assumption that all chloride is extracellular If 4 or 5 mEq of chloride are put into the cells essentially the same figures are obtained

What evidence is there for this relationship *in vivo* using the data available in muscle analyses? With increase or decrease in extracellular potassium concentration intracellular potassium seems to change according to the ratio, with appropriate changes in the chloride ratio However, this statement involves some uncertainty as to intracellular chloride concentration And the bicarbonate concentration in the cells cannot be reconciled with the theoretical ratio, even approximately This means that the hydrogen ion ratio is also different The probable potassium ratio demands a concentration of bicarbonate in the cells of something less than 1 mEq per liter of water Conway, of course, thinks that this concentration is probably right, and he defends his view by determining the barium insoluble bicarbonate in muscle, but I have never been quite sure that it is reliable Conway gets figures of the right order of magnitude, by this method I know Dr Wallace wouldn't think very much of it, because he determined the total carbon dioxide of the muscle and I think his figures come to around 10 to 12 mEq per liter of cell water

Wallace In normal muscle, per Kg of water

Darrow Is that for the muscle water or for the intracellular water?

Wallace Intracellular, corrected to a water basis

Darrow Now, if Conway's idea of an extremely low bicarbonate is true, it would require a pH in the cells of around 5 95, which I think is lower than most people believe I don't believe that Dr Wallace would defend the idea that all of the carbon dioxide liberated by acid is derived from bicarbonate, but I think that most of it is If you use his figure I think it would yield a pH somewhat higher 6 1 to 6 2

Wallace It is higher than that 6 9

Darrow I think that is probably a little too high

Wallace One trouble with either the pH we calculated, or the one that Conway did is that according to Faurholt (13) no carbamino carbon dioxide can exist at either of these values Conway points this out in his paper and is very careful not to call it carbamino

Darrow Well, I think that 6 9 is a little too high a pH I think the pH of the cells probably approximates 6 4

Editor's Note — Dr Darrow wishes to add the following Conference "afterthought

The discrepancy in Wallace's and my statements is accounted for by the amounts of carbon dioxide assumed not to be bicarbonate

Wallace I should like to repeat that there must be a very peculiar compound to make carbamino carbon dioxide in appreciable quantity at pH 6 2

Darrow I am getting way over my head What I want to suggest is that the whole idea of using the Donnan equilibrium involves two difficulties (*a*) the sodium concentration within the cells which can be said to be outside a diffusion equilibrium and also I think (*b*) the bicarbonate concentration within the cells Our problem is to develop some theory to explain intracellular sodium and bicarbonate concentrations Whatever theory we develop we have to deal with the facts that are described in the Donnan equilibrium We have to treat the changes in composition of cells in terms of ionic and osmotic forces There must be electric neutrality and osmotic equilibrium. Some ions freely diffuse and will respond promptly to changes in reaction or osmotic pressure independent of energy production by cells

TABLE XI

	$\frac{(HCO_3)_c}{(HCO_3)_e}$	pH_c	pH_e	B_c+	B_e+
Normal Animal	0 40	6 98	7.38	204	153
Potassium Deficient Animal	0 16	6 63	7 44	188	155

Average values for calculated bicarbonate ratios between muscle cell water and serum water calculated pH of cellular water (pH_c) serum pH (pH_e) total base concentration in cellular water (B_c+) and extracellular total base (B_e+) in normal and potassium deficient rats

From Schwartz R and Wallace W M Unpublished data

Wallace Table XI summarizes some data we obtained on rats made potassium-deficient very much as Dr Darrow has just described but without the administration of desoxycorticosterone The normal values are shown at the top of the Table The calculated distribution ratio for bicarbonate is 0 4 This is calculated assuming that all of the carbon dioxide is bicarbonate The extracellular pH in the normal animals is 7 38 The total base concentration in intra cellular and extracellular water is shown The calculations are made on the assumption that all chloride is extracellular If 4 or 5 mEq of chloride are put into the cells essentially the same figures are obtained

In the potassium deficient animals, the ratio is much lower. The calculated intracellular pH is lower, whereas the extracellular pH is higher, than in the normals The intracellular base concentration is greatly reduced The reduction in total base immediately points to a reason for the low pH without invoking any change in carbon dioxide tension One should note that the extracellular total base *concentration remains normal*

I should like to see whether Dr Steinbach has anything to say about this In some of his papers he talks about the fallacy of considering cells as 'empty sacks" This type of calculation does make the assumption that a cell is just an empty bag containing ions in solution Isn't it really compartmentalized? Is it reasonable to make this type of calculation? Dr Steinbach, I am trying to get you into this argument

I wonder whether you can think of the inside of a muscle cell as an empty sack,' as you have put it, that is, merely a bag in which *ions are floating around equally in all of the phases? Don't the A* and I discs of muscle cells have different concentrations of potassium? I think that some calculations made on the basis of muscle bicarbonate concentrations measured in rats made potassium deficient would indicate a potassium concentration, in some of the specialized phases of muscle cells as high as 450 mEq per kilogram

Steinbach The sad thing is that although the muscle is not an "empty sack,' if you go through a series of calculations assuming different degrees of ion partition within the muscle fiber, and then add up the fractions to give the whole muscle fiber, you come out *with about the same figure you would have had had you started* with an empty sack Usually one might as well assume a uniform intracellular distribution of ions, even though one doesn't believe it is true

Darrow Well, isn't it perfectly rational to do that? If it is unevenly distributed, it will be compensated by some other forces within the cells, so that you can consider it for practical purposes as an empty sack

Steinbach Yes, that s right

Darrow I should like to add to Dr Wallace's figures here that if you calculate the normal ratio of potassium concentration in the cells to that of extracellular fluids, it will be on the order of 40, and in the potassium deficient animals it will be somewhat higher, in other words, although the pH is not what Conway predicted, it goes in the same direction as the change in *R I have run through a* number of different calculations, and the change in (pHe - pHc)

follows the log R as indicated by the potassium ratio The change in the potassium ratio predicts the expected change in cell pH but the potassium ratio does not predict what we believe to be the correct cell pH

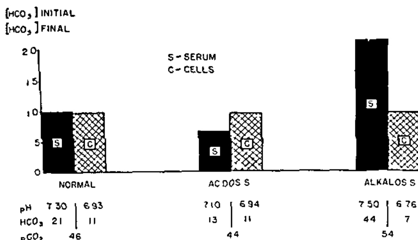


FIGURE 20 Ratio of initial to final HCO_3 concentrations in muscle and serum water in cats made acidotic and alkalotic by the administration of HCl and $NaHCO_3$ solutions Reprinted by permission from Wallace W M and Hastings A B Distribution of bicarbonate ion in mammalian muscle *J Biol Chem* 144, 637 (1942)

Wallace The last experiments were long term ones If the acid base disturbance is produced acutely, the relationship shown in Figure 20 is found The cross hatched areas represent the ratio of initial intracellular bicarbonate concentration to the final concentration after the acid base disturbances have been produced The black bars represent the same thing for the serum concentrations The calculated values for bicarbonate and pH are given at the bottom of the Figure These experiments were carried out in much the same fashion as those of Drs Pitts and Swan The muscle measurements were made during the control period and after the induction of acidosis and alkalosis The main point of the Figure is to show that in acute experiments muscle bicarbonate remains pretty constant even though extracellular pH is markedly changed The changes in calculated intracellular pH are almost entirely accounted for by the changes in carbon dioxide tension I might say that these experiments were done 12 years ago, at a time when

Berliner Would you like to estimate how much energy would be required to maintain the osmotic pressure of cells at twice that of the extracellular fluid, if the turnover time for labeled water is approximately a couple of minutes?

Ussing That is a question which may be more difficult than one realizes at once, because we have to deal with two types of permeability to water The rate of diffusion of isotopic water, like the diffusion of any molecular species, is determined by the total area available to diffusion The rate of osmotic flow, on the other hand is determined not only by the area but also by the size of the pores making up this area We are thus dealing with two theoretically different permeabilities one which one might call diffusion permeability, and another which one might call filtration permeability Prescott and Zeuthen (16), in our laboratory, have made measurements on different types of eggs They found that the two water permeabilities would nearly coincide only in very tight eggs That tends to show that if there are pores present in the membrane of these eggs, these pores must be very small With ovarian eggs of the frog, on the other hand, the two water permeabilities differ widely, so that the filtration permeability is about twenty times higher than the diffusion permeability expressed in the same units, for instance cm per second Therefore experiments with isotopic water alone do not give a measure of the rate of osmotic flow that one would encounter And of course, it is the osmotic stream rather than the diffusion stream which has to be counteracted by metabolic activity

REFERENCES

1 LIGHTFOOT, L H and COOLIDGE T B The distribution of collagen in the guinea pig *J Biol Chem* 176, 477 (1948)

2 COTLOVE E C Inulin and chloride space in muscle *Federation Proc* 11, 28 (1952)

3 FRIEDMAN, M M Simplified bromide determination in blood and urine *J Biol Chem* 144, 519 (1942)

4 WEIR E G Determination of the total chloride content of animals following administration of sodium bromide *Am J Physiol* 127, 338 (1939)

5 MOORE F D Determination of total body water and solids with isotopes *Science* 104, 157 (1946)

6 WINKLER A W ELKINTON J R, and EISENMAN A F Comparison of sulfocyanate with radioactive chloride and sodium in the measurement of extracellular fluid *Am J Physiol* 139, 239 (1943)

7 BURCH, G E, THREEFOOT, S A, and RAY, C T Rates of turnover and biologic decay of chloride and chloride space in the dog determined with the longlife isotope, Cl^{36} *J Lab & Clin Med* 35, *331 (1950)*

8 MOORE, F D, and BALL, M R *The Metabolic Response to Surgery* Springfield, Ill, Thomas, 1951 (p 126)

9 SINGER, R B, CLARK, J K, CROSLEY, A P, JR., and BARKER, E S Distribution of infused sodium bicarbonate solution in man *Federation Proc* 11, *148 (1952)*

10 DARROW, D C, SCHWARTZ, R, IANNUCCI, J F, and COVILLE, F Relation of serum bicarbonate concentration to muscle composition *J Clin Investigation* 27, *198 (1948)*

11 COOKE, R E, COUGHLIN, F R, JR, and SEGAR, W E Muscle composition in respiratory acidosis *J Clin Investigation* 31, *1006 (1952)*

12 BOYLE, P J, and CONWAY, E J Potassium accumulation in muscle and associated changes *J Physiol* 100, *1 (1941)*

13 FAURHOLT, C Études sur les solutions aqueses d anhydride carbonique et d acide carbonique *J Chim Phys* 21, *400 (1924)*

14 ROBINSON, J R, and MCCANCE, R A Water metabolism *Ann Rev Physiol* 14, *115 (1952)*

15 FRANCK, J, and MAYER, J E An osmotic diffusion pump *Arch Biochem* 14, *297 (1947)*

16 PRESCOTT, D, and ZEUTHEN, E Comparison of water diffusion and water filtration across cell surfaces *Acta physiol scandinav* 28, *77 (1953)*

occur The slices were incubated in Warburg vessels The medium had an initial potassium concentration of 5 mEq per L and contained small amounts of phosphate buffer calcium chloride acetate as substrate and sodium chloride up to isotonicity After incubation the slices were removed and analyzed directly for their *electrolyte* content In each experiment the simultaneous oxygen uptake was measured

At 25° and under optimal conditions the incubation of the tissues is associated with an uptake of potassium against a concentration gradient so that the final composition is similar to that of the fresh tissues Experiments under a variety of conditions have shown that this accumulation occurs only when there is simultaneous aerobic oxidation Thus without the addition of substrate there is a lower respiratory rate and likewise a depression of potassium uptake When aerobic metabolism is inhibited there is essentially no potassium accumulation This was shown in the experiments in which incubation was carried out at 2° C when the cups were gassed with *nitrogen instead of oxygen and also when respiration was depressed* by inhibitors such as cyanide

We studied about fifty metabolic inhibitors A few are summarized here (Figure 21) Mercury was of interest because of its pharmacologic action The only point I wish to emphasize is that it is possible to work with a concentration of mercurial which has no effect on the *q*O but which has a marked effect on electrolyte composition 6063 is an inhibitor of carbonic anhydrase and has no effect in our system Davies (2) from Krebs laboratory has reported a positive effect in a similar type of study I don t understand why there should be this discrepancy in the results

Taggart I think that the situation in Krebs and Davies experiments was a little different in that the slices were not initially as *potassium depleted As I recall from a conversation with* Krebs last year the tissue level of potassium often got back to the normal range but isotope studies showed the turnover to be low

Mudge Net exchange occurs quite rapidly so that a constant value is reached within about 30 minutes Although we regularly *used acetate* as a substrate there is nothing specific about it as any substrate that is readily oxidized such as members of the citric acid cycle has a similar effect on potassium uptake

Let us now consider a group of drugs which are of major interest because of their effect on certain intermediary reactions of metabolism In the experiment shown in Table XII we studied seven different nitrophenols These are some of the compounds which

TABLE XII

Effect of Substituted Phenols on Electrolyte Composition of Kidney Slices

Inhibitor	Conc	qO_2	Tissue		
	× 10^{-3} M		H_2O	K	Na + K
			%	mEq/kg	Wet
Initial value	—	—	78 5	82 9	143
Control	—	0 88	77 1	67 5	145
2-nitrophenol	0 025	0 81	76 5	68 8	146
4-nitrophenol	0 025	1 00	77.2	70 7	146
2 amino-4-nitrophenol	0 025	0 91	77.5	66 4	149
2 4,6-trinitrophenol	0 025	0 92	76 9	73 2	143
2 4 dinitrophenol	0 025	1 20	80 0	39 4	144
2,4 dinitro 6 phenylphenol	0 025	0 95	80 1	39 9	142
2,6-dichloro-4 nitrophenol	0 025	1 12	80 0	43 6	144

Reprinted by permission, from Mudge, G H Electrolyte and water metabolism of rabbit kidney slices effect of metabolic inhibitors *Am. J Physiol* 167, 206 (1951)

were reported by Cross *et al* (3) in their studies on aerobic phosphorylation They showed that the first four compounds are inactive, while the last three are active That is to say, the last three, including 2,4-dinitrophenol, at these concentrations and in a cell free system, do not depress the rate of oxygen uptake, but do prevent the generation of energy-rich phosphate compounds The first four, the inactive ones, have absolutely no effect on potassium uptake, while the last three, the ones which uncouple oxidative phosphorylation, inhibit the accumulation of potassium We conclude, therefore, that the uptake of potassium is in some way related to the production of compounds containing energy-rich phosphate bonds I may point out that in the experiment pictured in Table XII we had a very low concentration of inhibitor More striking effects are obtained at higher concentrations, and in this experiment (Figure 22) we have progressively increased the concentration of 2,4-dinitrophenol There is a graded effect on tissue electrolytes Note the respiratory stimulation that occurs within this critical range, which confirms

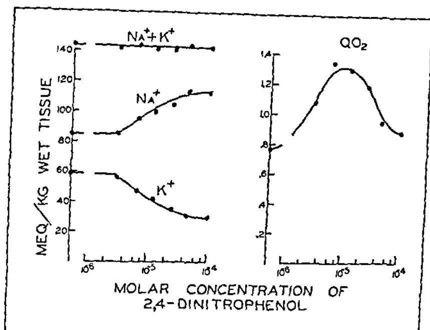


FIGURE 22 Effect of increasing concentrations of 2,4 dinitrophenol on electrolyte composition and oxygen consumption of kidney slices

the previous observations of many others We shall return to the subject of the nitrophenols later

I think that in outlining the general metabolic conditions required for potassium accumulation in this sort of system, it may be said (*a*) that it requires oxygen uptake, that is, active oxidation, (*b*) that it is not substrate specific, and (*c*) that the active nitrophenols, which uncouple phosphorylation, somehow interfere with the mechanism

Now, in terms of mechanisms postulated in the literature, one suggestion is that electrolyte transport may be related to ionic imbalance secondary to oxidation reactions occurring through the cytochrome system The results with the nitrophenols cannot be explained by this hypothesis, since potassium uptake is depressed while respiration is stimulated Another hypothesis is that the rate at which carbonic acid is hydrated is a determinant in cation transport, but, as I said before, we could find no evidence for this The mechanism which still appears most inviting is that of a carrier complex At one of three stages, i e, in forming or dissociating the

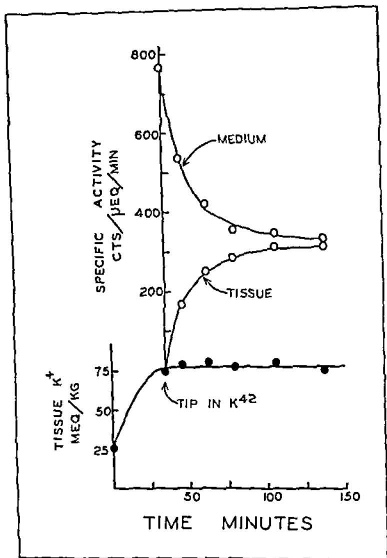


FIGURE 23 Equilibration of K^{42} with potassium of kidney slice

complex or in regenerating the carrier we have to put in some energy which is mediated through phosphate bonds

Let us now turn to an examination of the dynamic aspects of the slice system when studied with isotope techniques (Figure 23) The general experimental procedure was as follows The slices were incubated in oxygen and rapidly accumulated potassium so that a steady state was reached in about 30 minutes At this time a tracer amount of K^{42} was tipped in from the side arm The cups were then removed at intervals and the tissue and the medium from each cup were each analyzed for potassium and for radioactivity The chemical analyses showed that the tissues were in a so called steady state The changes in specific activity clearly showed that there was a rapid exchange of potassium between tissue and medium Under these conditions exchange was complete within about 60 minutes

When the results were plotted on semi log paper it was found that in no instance did they fit a single straight line The points are most simply described by two straight lines Without going into any mathematical calculations it is clear that there are two potassium components in the tissues accounting for about 32 and 68 per cent of the tissue K If we assume that the slices have an extracellular space of 40 per cent then only 2 5 per cent of the slice potassium is in the extracellular phase It is obvious that neither of the fractions can be identified with the extracellular phase We conclude therefore that intracellular potassium is not homogeneous

To study the role of metabolic activity we compared aerobic and anaerobic incubation (Figure 24) As noted previously the anaerobic tissue fails to accumulate potassium whereas with oxygen high values are observed Also as noted above potassium exchange in oxygenated tissue is rapid and complete However note that exchange in a nitrogen atmosphere is incomplete at 60 minutes and there is essentially no further exchange for as long as four hours In 28 anaerobic experiments 55 per cent of the tissue potassium exchanged within 60 minutes Thus in nitrogen about 45 per cent of the tissue potassium probably is nonexchangeable

Visscher Completely nonexchangeable?

Mudge I am coming to that Within the experimental error of the method it does not exchange Comparison of the results at 60 and 120 minutes shows that the difference is very small and not significant For practical purposes it does not exchange but the same potassium completely exchanges in oxygen

Visscher Well I think it is perfectly fair to say that the aerobic

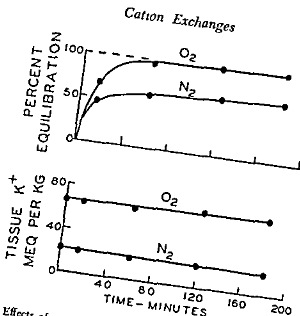


FIGURE 24 Effects of anaerobic and aerobic incubation on K exchange of kidney slices

situation greatly increases the rate of exchange There is no question about it from your data I don't mean to press this point now, but it may be of some interest later.

Mudge It may be of considerable interest Furthermore, I don't think there is any way of solving the problem from this type of experiment, that is, it does not tell us whether potassium exchanges very slowly or does not exchange at all We are dealing with a tissue which is normally aerobic, and I doubt whether anaerobic incubation for longer periods of time would yield very meaningful results

Visscher Don't you think it is equally interesting that a certain fraction of this exchange does occur so rapidly in nitrogen?

Mudge Yes, I do With that in mind, we analyzed the results in terms of the intra- and extra-cellular spaces In the experiments with nitrogen total tissue potassium was 18 mEq per kilogram Knowing the concentration in the medium, we can estimate that 3 mEq were extracellular, and 15 mEq intracellular Forty-five per cent of the potassium in the whole tissue is nonexchangeable under anaerobic conditions This represents about 8 mEq per kilogram Thus in the intracellular phase, we have 15 mEq of potassium, of which 8 mEq do *not* exchange, and 7 mEq which

arrive at a conclusion similar to that reached previously, namely, that intracellular potassium is not homogeneous Under anaerobic conditions it consists of an exchangeable, and a nonexchangeable fraction

Lotspeich Couldn't that difference between the exchange in nitrogen and oxygen be roughly of the same order as the phosphate bond energy, which is formed by the anaerobic and aerobic metabolism?

Taggart Perhaps we are on shaky ground, but it has certainly been our impression that anaerobic glycolysis makes a minimal contribution of energy to the work of the kidney In both the p aminohippurate and potassium transport systems, glucose shows no effect beyond that which is observed in the complete absence of added substrate In addition, we have measured the rates of utilization by kidney slices of glucose and certain members of the citric acid cycle, such as pyruvate and α ketoglutarate The latter disappear rapidly, but glucose utilization is so slow as to be almost undetectable There is no doubt that essentially all of the enzymes of the anaerobic glycolytic system can be demonstrated in kidney breis but I doubt that they are quantitatively very important

Mudge In reference to Dr Lotspeich's questions, we did some experiments in which a set of anaerobic tissues was compared to another anaerboic set to which iodoacetate had been added They were both the same, so I don't think we can attribute the exchangeable moiety to an anaerobic pump

Ussing Have you seen the paper by Harris (4), recently published in which he showed that muscle kept in potassium phosphate will not exchange a certain fraction of its potassium at zero degrees? I think that it is similar to your finding

Mudge Unfortunately, I have not had a chance to see his paper

Ussing On the other hand, you have cells with different functions so it might be that the fractions you get are not due to two types of potassium in the same cell, but rather to two types of cells which react differently

Mudge Yes, that problem has bothered us I am sure that it would make an enormous difference in the final interpretation of kinetic data, but I am not so sure that the differences in cell types constitute a stumbling block in the understanding of biochemical mechanisms Whether we consider a homogeneous or a heterogeneous population of cells the results indicate that the exchangeability of some of the potassium is metabolically determined, and that is the problem of immediate interest

We were also interested in the question of whether or not the

effects of anaerobic incubation are reversible Slices were regassed with oxygen after they had been incubated in nitrogen for as long as two hours, they behaved in a manner exactly similar to those which were in oxygen continuously This applies to net accumulation as well as to the rate of exchange The stability of the potassium transport system is quite remarkable Without presenting the experimental data, I should like to add that the amount of non-exchangeable potassium appears to be quite constant, and independent of the concentration in the incubation medium

I should now like to consider a question which has often been raised, namely, whether potassium exchanges primarily, or whether its movement is secondary to the migration of sodium To demonstrate that the exchange of one ion is secondary to the movement of another, *it* seemed to us that we would have to show first, that the movement of each ion is determined by the same metabolic reactions, and secondly, that the exchange or concentration of one is a limiting factor for the movement of the other

As to the first condition, Figure 25 shows the exchange of sodium

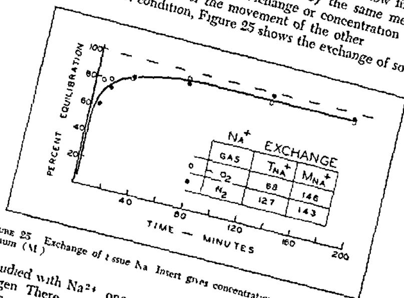
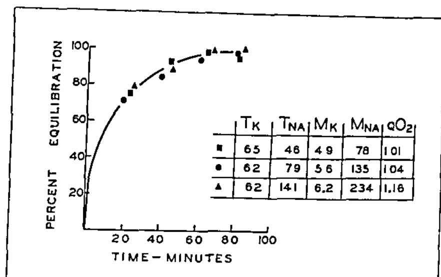


FIGURE 25 Exchange of tissue Na Insert gives concentrations for tissue (T) and medium (M)

as studied with Na^{24} one set of tissues in oxygen, and another in nitrogen There is really no difference in the early part of the two curves, and certainly the latter parts are identical Thus sodium exchange is very different from potassium, and shows no dependence on aerobic metabolism As to the second condition, the pertinent data are in Figure 26 All tissues were in oxygen and the



	T_K	T_{NA}	M_K	M_{NA}	QO_2
■	65	46	4.9	78	1.01
●	62	79	5.6	135	1.04
▲	62	141	6.2	234	1.16

FIGURE 26 Effect of Na concentration on K exchange in kidney slices

variable factor was the amount of sodium added to the medium (M_{NA}) from 78 to 234 mEq per liter Within this range the oxygen quotients (qO_2) are constant Tissue potassium (T_K) was likewise constant, and all tissues equilibrated at the same rate Thus there is no evidence that the sodium concentration is a limiting factor for potassium exchange It is to be recalled from the earlier Figures that so far as net changes are concerned, sodium and potassium reciprocate However, from these last two Figures we conclude that the movement of potassium into and out of the cell is quite independent of the movement of sodium

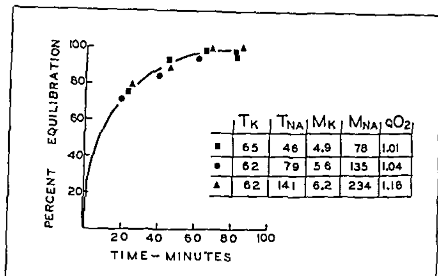
I think that the important points of the slice experiments with K^{42} are first, that intracellular potassium is nonhomogeneous, and second, that there is a potassium component which anaerobically is virtually nonexchangeable but which rapidly exchanges under conditions of active metabolism Taken together, these observations may mean that some components within the cell have a specific potassium metabolism characterized by the active formation or dissociation of relatively stable potassium complexes represented by KZ in our hypothesis We therefore set out to study potassium transport in a cell free system using liver in the hope of finding chemical reactions which could be more precisely defined

I should like to explain why we switched from kidney to liver In the slice system kidney is definitely superior since slices from

liver are very fragile and deteriorate rapidly In the cell free system on the other hand liver appears to be better We required fairly large quantities of material and liver is obviously a better starting point In addition the fragility of the liver cell makes it easier to obtain a good yield of viable mitochondria We originally planned to do some preliminary studies on liver and then return to the kidney These preliminary studies are still in progress However in so far as the two systems have been directly compared — that is slices and mitochondria from both kidney and liver — we found that they were qualitatively similar even though definite quantitative differences may be found in the future

At this point in the work I was very fortunate in being joined by Dr S W Stanbury of the University of Manchester who was with us for a year on a Rockefeller Fellowship I am sorry that he has returned home and that he was unable to be at this meeting The reasons we turned to the mitochondrial fraction may be summarized as follows (*a*) As studied in the slices potassium transport depends on aerobic oxidation and associated phosphorylation reactions Since the early studies of Hogeboom and Schneider (5) it has been repeatedly confirmed that these reactions may be localized for the most part to the mitochondrial fraction of tissue homogenates (*b*) We found evidence relating to the phenomenon of potassium binding and demonstrated that the exchangeability of this fraction was metabolically determined No data were available on the potassium metabolism of mitochondria (*c*) For what it is worth we thought we should look for something that had some sort of a spatial orientation within the cell Dr Stanbury and I prepared mitochondria from the liver by a modification of the technique described by Lehninger (6)

Oliver I think this is often done The liver is the usual source of mitochondria because from the morphologist's standpoint the cytoplasmic structures are a heterogeneous mixture They are all sorts of things even including such indeterminate objects as secretion granules although no one has any idea what secretory process is involved One doesn't know therefore what one is dealing with and this is recognized by the fact that many workers hesitate to use the word mitochondria at all and refer to the fraction as composed of large granules" In the kidney one can be more certain of dealing with one sort of morphological unit because the rodlets which form the great bulk of the mitochondria in the cortex are specific characteristic objects quite apart from their spatial orientation They can be recognized whereas with the so called "mito



	T_K	T_{NA}	M_K	M_{NA}	qO_2
■	65	46	4.9	78	1.01
●	62	79	5.6	135	1.04
▲	62	141	6.2	234	1.16

FIGURE 26 Effect of Na concentration on K exchange in kidney slices

variable factor was the amount of sodium added to the medium (M_{NA}) from 78 to 234 mEq per liter Within this range the oxygen quotients (qO_2) are constant Tissue potassium (T_K) was likewise constant, and all tissues equilibrated at the same rate Thus there is no evidence that the sodium concentration is a limiting factor for potassium exchange It is to be recalled from the earlier Figures that so far as net changes are concerned, sodium and potassium reciprocate However, from these last two Figures we conclude that the movement of potassium into and out of *the cell* is quite independent of the movement of sodium

I think that the important points of the slice experiments with K^{42} are first, that intracellular potassium is nonhomogeneous, and second, that there is a potassium component which *anaerobically* is virtually nonexchangeable but which rapidly exchanges under conditions of active metabolism Taken together, these observations may mean that some components within the cell have a specific *potassium metabolism characterized by the active formation* or dissociation of relatively stable potassium complexes represented by KZ in our hypothesis We therefore set out to study potassium transport in a cell free system using liver, in the hope of finding chemical reactions which could be more precisely defined

I should like to explain why we switched from kidney to liver In the slice system, kidney is definitely superior since slices from

liver are very fragile and deteriorate rapidly In the cell free system on the other hand liver appears to be better We required fairly large quantities of material and liver is obviously a better starting point In addition the fragility of the liver cell makes it easier to obtain a good yield of viable mitochondria We originally planned to do some preliminary studies on liver and then return to the kidney These preliminary studies are still in progress However in so far as the two systems have been directly compared — that is slices and mitochondria from both kidney and liver — we found that they were qualitatively similar even though definite quantitative differences may be found in the future

At this point in the work I was very fortunate in being joined by Dr S W Stanbury of the University of Manchester who was with us for a year on a Rockefeller Fellowship I am sorry that he has returned home and that he was unable to be at this meeting The reasons we turned to the mitochondrial fraction may be summarized as follows (*a*) As studied in the slices potassium transport depends on aerobic oxidation and associated phosphorylation reactions Since the early studies of Hogeboom and Schneider (5) it has been repeatedly confirmed that these reactions may be localized for the most part to the mitochondrial fraction of tissue homogenates (*b*) We found evidence relating to the phenomenon of potassium binding and demonstrated that the exchangeability of this fraction was metabolically determined No data were available on the potassium metabolism of mitochondria (*c*) For what it is worth we thought we should look for something that had some sort of a spatial orientation within the cell Dr Stanbury and I prepared mitochondria from the liver by a modification of the technique described by Lehninger (6)

Oliver I think this is often done The liver is the usual source of mitochondria because from the morphologist s standpoint the cytoplasmic structures are a heterogeneous mixture They are all sorts of things even including such indeterminate objects as secretion granules " although no one has any idea what secretory process is involved One doesn t know therefore what one is dealing with and this is recognized by the fact that many workers hesitate to use the word mitochondria at all and refer to the fraction as composed of "large granules In the kidney one can be more certain of dealing with one sort of morphological unit because the rodlets which form the great bulk of the mitochondria in the cortex are specific characteristic objects quite apart from their spatial orientation They can be recognized whereas with the so called "mito

chondrial fraction from the liver one cannot be sure of what one is working with

Taggart But in contrast to that I think everybody who has made sucrose preparations attempting to isolate mitochondrial fractions has found that the kidney is a rather difficult organ to deal with because one ends up almost invariably with a considerable contamination of the nuclear fraction with mitochondria

Oliver Not everybody the biochemists in our laboratory get their fractions out reasonably clean

Taggart From the enzymologist s standpoint if one tries to fractionate and get clean cut distribution of activities in one fraction or another it is fairly easily achieved with liver With kidney I don t know of anyone who has succeeded You have dealt with it from the morphological standpoint we have dealt with it from the standpoint of enzymatic activities and we have always had to conclude that in dealing with kidney fractions we were obviously dealing with badly contaminated fractions

Oliver Do you think it may be that the enzymes are distributed differently in the kidney fractions? That is they may not be so *specifically localized as they are in other organs such as the liver*

Taggart All one has to do is look through the microscope to see Janus green material in handling the nuclear fraction One can find DNA in the mitochondrial fraction

Oliver We did not find DNA in our mitochondrial fractions that is a routine we use to check on nuclear contamination

Mudge There are some very practical points involved Since we needed large amounts of material we decided to do our preliminary studies on liver and I suppose these may go on for a long time It was also clear that we were dealing with a fairly labile material In starting with kidney one must homogenize a lot longer and I am not at all sure that the activity we are studying would survive the more vigorous procedure

Liver mitochondria were prepared by a modification of the technique described by Lehninger All steps were carried out in the cold at approximately 4° C The initial homogenate was prepared in 8 5 per cent sucrose The nuclear fraction and other heavy particles including intact cells were centrifuged down at low speeds and were discarded Enough 1 N NaCl was then added to the supernatant to make it isotonic with reference to NaCl The mitochondrial particles were then centrifuged down and were repeatedly washed in cold sodium chloride The potassium which was found in the mitochondria is shown in Figure 27 All values were referred

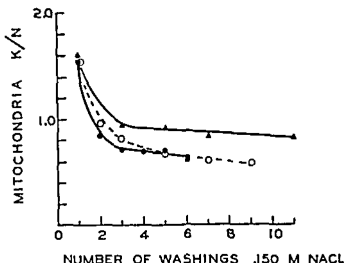


FIGURE 27 K/N ratio of liver mitochondria during successive washes during preparation

to nitrogen as a reference point As the mitochondrial fraction was washed with cold sodium chloride, the amount of potassium initially fell abruptly, but then changed very little with repeated washings In 50 experiments after three washings, the K/N ratio was about 0 8 mEq per gram The results clearly indicate that potassium adheres to the mitochondria so that it cannot be washed off

The question may be immediately raised as to just how much potassium from the intact cell can be accounted for in the mitochondrial fraction We estimate that it is at least 1 8 per cent The exact figure would be larger, since we do not recover the mito chondria quantitatively

The standard experiment was carried out with mitochondria which had been washed three times They were added to Warburg cups containing the desired components, and were usually incubated at 25° for one hour The cups were then decanted into a large vol ume of cold sodium chloride solution, and the mitochondria were washed three times so as to remove potassium derived from the incubation medium They were then analyzed for electrolyte and nitrogen

Figure 28 shows the results obtained under optimal conditions In addition to 1 ml of mitochondria, each cup contained 30 micro moles of α ketoglutarate as substrate KCl in a concentration of 25

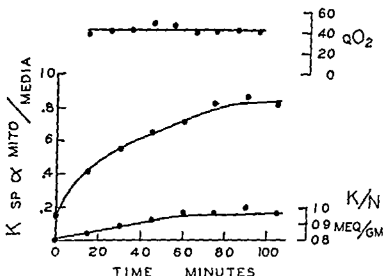


FIGURE 28 Exchange of mitochondrial K with medium K during aerobic incubation, 25° C

mEq per L, a trace amount of K^{42}, tris buffer at pH 7 4 and a small amount of magnesium I wish to emphasize several points from the Figure Note that the K/N ratio is fairly constant and as a matter of fact, in this experiment it rose slightly Note also that the qO_2 is constant during the period of incubation And finally note that under these conditions of active metabolism the potassium of the mitochondria exchanges with that of the medium

The K/N ratio is maintained during incubation at 25° only when the mitochondria are metabolically active They lose some of their potassium when there is no added substrate, or when they are incubated anaerobically I think that at this point we can safely say that we have answered two questions which we previously raised concerning the mitochondria first, they appear to contain potassium and secondly, to a certain extent we can correlate the amount of this potassium and its exchangeability, with metabolic activity

The experiment depicted in Table XIII pertains to the general nature of the mitochondrial potassium The mitochondria were incubated as previously but when they were harvested" one half of the cups were washed with isotonic KCl while the other half were treated as usual with isotonic NaCl The enzyme that was washed in NaCl contains the usual amount of potassium as deter

TABLE XIII

	Treatment of Mitochondria after Incubation			
	Washed in NaCl			Washed in KCl
Exp	K/N	Sp Activity Ratio	K^{42}	K^{42}
	mEq /Gm	mito/media	cts/mg N	cts/mg N
1	1 02	0 82	136	104
2	0 81	0 71	152	154

mined chemically We are of course unable to do chemical analyses for potassium on the enzyme which was washed with KCl, but note that the counts of K^{42} are the same for the two sets May I remind you that all washing procedures were carried out in the cold Clearly, mitochondrial potassium does not exchange with potassium from the washing solution In the absence of metabolic activity potassium appears to be quite firmly attached to the enzyme

Dr Stanbury and I explored a number of variables with this system, but I should like to devote the rest of my time to a series of reactions which, to us at least, seemed to be of the greatest interest, namely, the effect of phosphate metabolism on the behavior of potassium When we examined the effect of pH – which parenthetically is not very striking from about 7 0 to 7 5 – we used a tris buffer as well as a phosphate buffer, and much to our surprise obtained much lower values with phosphate As expected, phosphate stimulates respiration, yet at the same time greatly depresses the amount of potassium held by the enzyme This, apparently, is a specific effect It is not due to changes in osmotic pressure, since similar concentrations of sodium chloride, or glucose, are quite inert It is not the direct result of respiratory activity which can be in-

contained in the mitochondria themselves If I may return to the

kidney slices for a moment, I should like to point out that they work equally well with chloride, nitrate, or sulfate as the major anion of the medium, but that potassium accumulation is inhibited by phosphate

Lotspeich What was the final molar concentration of phosphate in the cups where there was the maximum inhibition of exchange?

Mudge Fifty per cent inhibition was noted with 5 μM per ml while the maximal effect was at about 15 μM per milliliter These values are the phosphate concentration at the start of incubation the final ones would be somewhat lower

When we studied dinitrophenol (DNP) we had reason to believe, from the work with the slices, that it would inhibit potassium uptake We also measured orthophosphate, because it was itself inhibitory, and because we had anticipated, from the work of others that its concentration would be increased by DNP

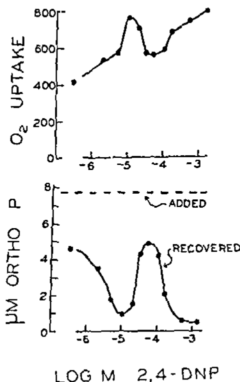


FIGURE 29 Effect of 2 4 dinitrophenol on respiration and amount of orthophosphate recovered after incubation of mitochondrial suspension

Let us leave the potassium problem for a moment and consider the phosphate effects Figure 29 shows the effect of DNP Now recall that no trapping mechanism has been added to this system that is no fluoride no hexose and no hexokinase The essential phosphate reactions are most readily studied with about 6 micro moles of orthophosphate added per cup In addition there is the phosphate from the mitochondria The action of dinitrophenol is a most peculiar one Depending on its concentration it apparently has three effects Control values are given at the left At about 10^{-5} M more orthophosphate disappears than in the control. At about 10^{-4} phosphate reappears or perhaps more exactly fails to disappear Then as very high concentrations of DNP are reached orthophosphate again almost completely disappears The unidentified phosphate ester of course behaves reciprocally It first increases then decreases and then finally increases again The upper graph (Figure 29) shows the effects on respiration As is well known dinitrophenol tends to increase oxygen consumption but the mechanism of this action has not been clearly established Without going into detail the results indicate a correlation between respiration and the concentration of phosphate acceptors However this is a very complex problem and despite the correlation depicted here there are definitely other variables of importance

As far as we know these effects of dinitrophenol have not been previously described I want to emphasize that they in no way disprove the conventional concept that dinitrophenol uncouples oxidative phosphorylation That is essentially a rate reaction studied with the addition of ATP fluoride hexokinase and hexose as the phosphate acceptor We are studying a net reaction without any added trapping mechanism At present our data simply indicate that dinitrophenol has an effect on certain phosphate fractions that has not been previously examined The interrelationship of these fractions is almost certainly very complex as is the effect of dinitrophenol upon them

I should like to emphasize a few of the points which have been established from a large number of experiments First the unidentified phosphate is present in very large quantities — up to 10 micromoles per cup in some experiments Secondly the distribution of phosphate on the washed enzyme parallels the concentration found on analysis of the contents of the whole cup And thirdly ortho-

phosphate disappears when α-ketoglutarate, or its precursors, are oxidized, but does not disappear with succinate I will not speculate on these three observations except to say that I think they are of some importance in attempting to characterize the phosphate compounds from a chemical point of view

Although these results with phosphate are in themselves very intriguing, they are of greater interest because of the simultaneous changes in potassium metabolism, as shown in Figure 30 The curve

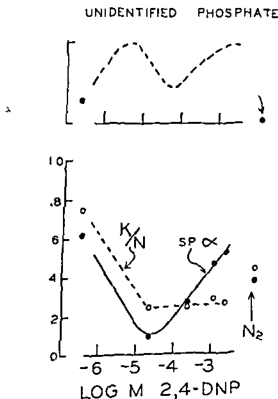


FIGURE 30 Effect of 2 4 dinitrophenol on level of mitochondrial K and its exchangeability Comparison is also made to a *incubation (withou* \P)

for phosphate is a schema tion of th ust described I have also added f the res under aerobic conditions There ry striki ut the behavior th mo ties of m The ich a to- mitroph ount o

chondria is very small a lot less than under anaerobic conditions At present we have no real explanation for this Note that the K/N ratios appear to approach a minimal value when the lost phosphate fraction is also at a minimum One is tempted to jump to the conclusion that this indicates the formation of a complex consisting of mitochondria potassium and some type of phosphate Absolute evidence for this interpretation has as yet not been obtained but the other results shown here (Figure 30) encourage us to explore this

drial potassium It is also in this range that there is an increase in the unidentified phosphate Under these circumstances the amount of sodium which we can find on the mitochondria is very small It has a low rate of exchange and does not quantitatively reciprocate with potassium

Before summarizing I should like to point out that the correlation between potassium and phosphate is not a simple or consistent one There are several apparent contradictions First at the low range of DNP phosphate increases yet the mitochondria lose potassium Secondly in nitrogen there is about half as much potassium in the mitochondria as in oxygen yet there is virtually no phosphate ester which we can detect And thirdly the potassium at 10^{-4} M DNP is a great deal lower than with anaerobic incubation For the moment the major point of interest is that at the high concentration (10^{-3} M) of DNP there is some sort of a correlation between potassium and anion and that there is enough of the anion so that it may be studied further

I see no way in which all the data which I have presented can be put together into any single scheme of transport of electrolyte or its intracellular metabolism For the time being all we can say is that data such as these must eventually be taken into account in any comprehensive analysis

Bott Perhaps this should be obvious but what is the pathway that you picture for your potassium when it leaves the cells? Do you think of it as being extruded in the way that the kidney ordinarily operates or is it simply diffusing out of these slices in some other way?

Mudge You mean is it coming out through the tubules to the lumen?

Bott Yes

Ussing Yes but then that does not prove that it is not an active transport

Mudge I cannot correlate the flux of sodium with any metabolic function No matter how the slices are incubated sodium movement is the same

Ussing What we mean is that it moves Do you mean that the exchange rate is the same?

Mudge Yes

Ussing Because sodium certainly gets out of the slice in oxygen

Mudge I am not referring to net changes which are clearly the opposite of potassium The sodium concentration in the slice is also directly dependent on the external concentration which is not true for potassium

Ussing That s right but on the other hand at equilibrium the potassium concentration inside the cell would depend upon the out side concentration I don t mean to imply that there really is any thing like a Donnan equilibrium here but on the other hand I do not think there is proof that sodium behaves passively in this system

Mudge Well that is a question I should like to raise Just what evidence does one need to prove that one is pumping substance A" in one direction or B in the opposite?

Ussing Or both The point I was trying to make is that in case the interior of the cell turns out to be positive relative to the outside then sodium extrusion may be only apparent and really only the result of inward transport of potassium On the other hand if the cell like most cells is negative inside then the sodium will have to pass outward not only against the concentration gradient but also against an electrical one Thus the work may be larger than it seems considering the concentrations

Mudge To me the disturbing problem is the nature of the work or the source of the energy In our studies sodium behaves the same in nitrogen as in oxygen if anything the actual sodium flux is greater anaerobically

Visscher Through different tracer experiments yes but it makes a big difference in the net movement

Ussing Oh yes

Pitts Does that mean then that in terms of this hypothesis you can t demonstrate any NaZ ?

Mudge In about 10 experiments 83 per cent of the tissue sodium exchanged with that of the medium That leaves a small *fraction* unaccounted for If this were due to analytical error I would expect results to vary around the 100 per cent mark It may *well be*

that some sodium is nonexchangeable We talked about KZ possibly there is an NaZ or an NaX Sodium is much harder to study in this respect than is potassium

Steinbach What is the per cent exchange of sodium and potassium at the initial stage as compared to the final? What is per cent ex change of low potassium and per cent exchange of high sodium (final condition) as compared with percentage exchange of the ions in the low sodium high potassium fresh condition? If you get apparently complete exchange of sodium in high sodium slices in the absence of oxygen and a 50 per cent exchange of potassium in high potassium slices then that means that many of the base binding groups have changed their characteristics during the process of sodium potassium transfer

Mudge Well the sodium is completely exchangeable no matter what

Steinbach Then you really have a problem haven t you? On the quantitative basis you have changed to a very considerable amount the base binding properties of the cell

Mudge I don t know You mean in terms of osmotic pressure?

Steinbach Yes or total ionic strength

Mudge The amount bound — we will say bound potassium — is 45 per cent under anaerobic conditions but that is only 8 per cent of the amount that is there aerobically

Steinbach That means all of your exchange data are on the sodium rich slices

Mudge Yes that s right

Steinbach Then perhaps there is an equivalent binding of sodium as well as potassium that would not show up on a percentage scale

Mudge On an equivalent basis there might be

Steinbach You have so much excess sodium present that a small nonexchangeable fraction would be completely masked

Mudge Yes that is true It is almost impossible to get reliable data on the apparently nonexchangeable sodium If this were a relatively fixed amount as in the case of potassium then we could make it more apparent by lowering the external sodium concentra tion When that is done the absolute amount in the tissue falls proportionately but the per cent of this which exchanges is just about the same as before

Steinbach You see the same thing applies to the mitochondria I have been doing a few rough calculations The amount of potas sium bound on the protein of the mitochondria is less than a tenth of the base-binding capacity of that protein assuming it is a normal

Ussing Yes, but then that does not prove that it is not an active transport

Mudge I cannot correlate the flux of sodium with any metabolic function No matter how the slices are incubated, sodium movement is the same

Ussing What we mean is that it moves Do you mean that the exchange rate is the same?

Mudge Yes

Ussing Because, sodium certainly gets out of the slice in oxygen

Mudge I am not referring to net changes which are clearly the opposite of potassium The sodium concentration in the slice is also directly dependent on the external concentration, which is not true for potassium

Ussing That's right, but, on the other hand, at equilibrium the potassium concentration inside the cell would depend upon the outside concentration I don't mean to imply that there really is any- [illegible] ther hand I do [illegible] in this system [illegible] aise Just what evidence does one need to prove that one is pumping substance "A" in one direction, or "B" in the opposite?

Ussing Or both The point I was trying to make is that in case the interior of the cell turns out to be positive relative to the outside, then sodium extrusion may be only apparent, and really only the result of inward transport of potassium On the other hand, if the cell, like most cells, is negative inside, then the sodium will have to pass outward, not only against the concentration gradient, but also against an electrical one Thus the work may be larger than it seems, considering the concentrations

Mudge To me, the disturbing problem is the nature of the work, or the source of the energy In our studies, sodium behaves the same in nitrogen as in oxygen, if anything, the actual sodium flux is greater anaerobically

Visscher Through different tracer experiments, yes, but it makes a big difference in the net movement

Ussing Oh, yes

Pitts Does that mean, then, that in terms of this hypothesis you can't demonstrate any "NaZ ?

Mudge In about 10 experiments, 83 per cent of the tissue sodium exchanged with that of the medium That leaves a small fraction unaccounted for If this were due to analytical error I would expect the results to vary around the 100 per cent mark It may well be

that some sodium is nonexchangeable We talked about KZ possibly there is an NaZ or an NaX Sodium is much harder to study in this respect than is potassium

Steinbach What is the per cent exchange of sodium and potassium at the initial stage as compared to the final? What is per cent exchange of low potassium and per cent exchange of high sodium (final condition) as compared with percentage exchange of the ions in the low sodium high potassium fresh condition? If you get apparently complete exchange of sodium in high sodium slices in the absence of oxygen and a 50 per cent exchange of potassium in high potassium slices then that means that many of the base binding groups have changed their characteristics during the process of sodium potassium transfer

Mudge Well the sodium is completely exchangeable no matter what

Steinbach Then you really have a problem haven t you? On the quantitative basis you have changed to a very considerable amount the base binding properties of the cell

Mudge I don t know You mean in terms of osmotic pressure?

Steinbach Yes or total ionic strength

Mudge The amount bound — we will say bound potassium — is 45 per cent under anaerobic conditions but that is only 8 per cent of the amount that is there aerobically

Steinbach That means all of your exchange data are on the sodium rich slices

Mudge Yes that s right

Steinbach Then perhaps there is an equivalent binding of sodium as well as potassium that would not show up on a percentage scale

Mudge On an equivalent basis there might be

Steinbach You have so much excess sodium present that a small nonexchangeable fraction would be completely masked

Mudge Yes that is true It is almost impossible to get reliable data on the apparently nonexchangeable sodium If this were a relatively fixed amount as in the case of potassium then we could make it more apparent by lowering the external sodium concentra tion When that is done the absolute amount in the tissue falls pro portionately but the per cent of this which exchanges is just about the same as before

Steinbach You see the same thing applies to the mitochondria I have been doing a few rough calculations The amount of potas sium bound on the protein of the mitochondria is less than a tenth of the base binding capacity of that protein assuming it is a normal

protein of equivalent combining weight of about 1000, so there again it would be worthwhile if you could work up some methods to find out what is happening to the other ions

Mudge We have a rough idea of what is happening to the sodium of the mitochondria, although analytically the results are less satisfactory than with potassium There is about one third as much sodium as potassium in the mitochondria This amount is quite constant It exchanges very slowly, it is not altered by oxygen tension, and it is not affected by dinitrophenol Of course, the sodium data are from experiments in which the mitochondria were washed with KCl

Steinbach Yes, but that is sodium plus potassium, each calculated on a nitrogen basis, which again is another relative figure, you see This means that under anaerobic conditions the mitochondria might just be 'leaking' protein in general, and there might be a perfectly reciprocal exchange on what is left

Mudge But the electrolytes are calculated on a basis of nitrogen that is, nitrogen actually recovered from the washed mitochondria of each cup

Steinbach If you put in 100 units of mitochondrial nitrogen at the start do you get 100 units of mitochondrial nitrogen back again?

Mudge We don't get it, we get about two thirds of it

Binkley I should like to suggest that one need not consider proteins alone in the binding of potassium Pentose nucleic acids will bind potassium and appear to bind potassium, in a specific fashion There may be, of course, no relationship to the accumulation of potassium by cells, but it is easily demostrated that pentose nucleic acids bind potassium in preference to sodium This preferential binding of potassium applies to only about one third of the total base binding capacity of the pentose nucleic acid Sodium in this position is replaced by potassium in media of neutral pH, whereas the reverse is not easily accomplished

Steinbach What sort of experiment is that? The reason I ask is that some old data of, I believe Hammerstein, might sometimes be interpreted as a binding of ions on nucleic acid I think it was sodium in that case, wasn't it?

Ussing Yes, rather than potassium

Binkley I should explain that these observations were made with pentose nucleic acids isolated from kidney tissue of the pig Enzymatic activity of these preparations was found to depend upon the presence of potassium and in fact, upon considerable care to insure the combination of the nucleic acids with potassium Thus

potassium may be removed and the activity destroyed by prolonged contact with 3 molar sodium chloride activity may be restored and potassium returned by contact with solutions of potassium chloride of concentrations of about 0 1 molar Thus the affinity for potassium is considerably greater than the affinity for sodium These statements apply to experiments in the physiologic range of pH i e about 7 At lower values as would be expected the affinity for potassium is decreased The binding of potassium by nucleic acids must be a property of the phosphate groupings

Steinbach You get a fair load of pentose acid in these mitochondria but there isn t too much nucleic acid at that Isn t it 60 per cent protein and about 30 lipid?

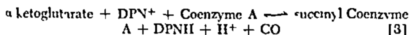
Oliver I never can remember the exact figures There is a considerable amount of phospholipid and very little pentose nucleic acid

Steinbach I think that s right

Oliver There is even some argument as to whether there is any PNA at all in the mitochondria and whether it may not be actually in microsomes that are absorbed on the surface of the mitochondria

Taggart Dr Binkley has brought the discussion to one of the features which interest us most While it is apparent that Dr Mudge started on a problem of potassium transport he has incidentally opened up some very interesting vistas in phosphorus metabolism I am not sure that he will ever get back to potassium again

One particular feature which should be emphasized is the difference between α ketoglutarate and succinate in promoting the esterification of orthophosphate Fortunately the reactions involved in this area of metabolism have been considerably clarified during the past year by Sanadi and Littlefield (7) and by Kaufman (8) The oxidation of α ketoglutarate proceeds as follows



$$\alpha \text{ ketoglutarate} + DPN^{+} + \text{Coenzyme A} \longrightarrow \text{succinyl Coenzyme A} + DPNH + H^{+} + CO \quad [3]$$

The question arises as to the relationship between succinyl Coenzyme A and phosphorylation mechanisms There is fairly good evidence that the succinyl group may be exchanged with orthophosphate to yield the corresponding phosphoryl Coenzyme A a form of energy rich phosphate Such a phosphate can in turn be transferred to ADP to yield ATP This mechanism however fails to account for the relatively large amounts of orthophosphate which disappear in the presence of 10^{3} M dinitrophenol Coenzyme A and the adenine nucleotides are present in Dr Mudge s system in far

too limited quantities to allow them to be the ultimate phosphate acceptors In fact there is no known phosphate acceptor present in sufficient quantity unless it be the substrate The idea of phosphorylated intermediates in the citric acid cycle was a popular one several years ago While it has fallen somewhat into disfavor more recently I suspect that it may now have to be revived Such phosphorylated intermediates may very likely be bound on the mitochondrial particles One wonders whether the potassium may be associated with such compounds The only other obvious explanation to account for the disappearance of orthophosphate might be the formation of some inorganic polyphosphate

Binkley Can it be metaphosphate? Have such tests been made?

Mudge I dont think it is a metaphosphate We suspect that it is a phosphorylated substrate but the evidence is far from complete

EDITORS NOTE — Dr Mudge wishes to add the following Conference afterthought

Subsequent experiments have shown that dinitrophenol in high concentrations apparently stimulates the formation of phospho enol pyruvate

Taggart Metaphosphate has been found in yeasts molds and insects and I am sure that there are some biochemists who harbor the conviction that it will eventually be found in the mammalian cell as well

Binkley Many biochemists also have the idea that there is no orthophosphate in tissues and that the material usually considered as orthophosphate is in reality a labile phosphate

Mudge This is not labile

Binkley Metaphosphate would satisfy the requirements would it not?

Mudge I believe that the hydrolysis characteristics are quite variable and depend on the metaphosphate in question When simple metaphosphoric acid is heated in acid it is quite rapidly converted to orthophosphate We have no data on other metaphosphates

Binkley Metaphosphate would be an ideal compound for it does appear to have specific binding properties

Visscher Dr Mudge what would be wrong with the view that what exists in these kidney slices is simply a stable potassium compound — one stable in nitrogen but going through some metabolic process in oxygen that there is a certain amount of transfer across the cells going on because there is leakage of potassium out all of the time and a return of some potassium from the medium

so that in the end result it is ionically active potassium which changes very quickly We could say that all of the sodium is essentially in an exchangeable position to the cell and that the really active process is the movement of sodium out which occurs only in the presence of oxygen and that this conditions the system so *that perhaps the accumulation of potassium which occurs in* oxygen might be due to electrical forces as other people have postulated? Why can t you accept the view that your unexchangeable potassium is simply a potassium store of some compound stable in nitrogen

Mudge You mean that the nonexchangeable potassium goes around in a little circle all by itself and that it has nothing to do with the major reactions of accumulation or transport?

Visscher Yes that is what I am suggesting that it is really an irrelevancy as far as accumulation is concerned

Mudge Well I just don t know As I said before I know of no way in which all the data can be put together in one single scheme

Visscher I can t see that anything you have told us fails to fit into such a simple view

Steinbach I can

Visscher How?

Steinbach You can t account for 50 per cent of the potassium that way

Visscher How do you mean?

Steinbach There isn t enough stuff known in the cell which might immobilize the potassium

Mudge But don t forget Dr Steinbach that is 45 per cent of a small amount of potassium That is the tissue level is low under anaerobic conditions

Steinbach Another objection I have is that to the best of my knowledge all so called bound ions which have been studied exchange very rapidly indeed *Isn t that true?*

Ussing Well I wouldn t say all

Steinbach If you take serum for example with bound calcium if you put in radioactive calcium you get rapid exchange I don t know of any type of ion binding other than a compartmentalization

up with

ould be

That is

e either

binding or transport

Visscher Well, maybe it is compartmentalized

Mudge I have been trying to find some data on the exchange ability of chelation complexes in pure systems — not in biological material I have not been able to find any reports on potassium

Binkley I believe another aspect should be emphasized One cannot devise a sodium pump without describing a system with a preference toward sodium as distinguished from other cations We must then speak of specific binding of sodium by a sodium pump and cannot, at the same time, use the idea of a sodium pump to rule out specific binding of cations as a partial explanation of the differential distribution of cations

Ussing Certainly The only difference is that it is possible to demonstrate the sodium pump in relatively pure form, whereas it is still a question whether in the present system there is a potassium pump, or whether we are dealing with a potassium inclusion in something Isn't that right?

Binkley But you are speaking of preference for sodium and not about absolute specificity in your experiments

Ussing Even if sodium and potassium were found in complex form, they would still probably exchange quite rapidly as Dr Steinbach said

Binkley Yes, under certain conditions exchange might be very rapid, and with other conditions, dependent upon the binding group, exchange would be slower

Ussing It might

Mudge From what I have been able to find, the problem of determining whether or not bound potassium is exchangeable is like trying to decide on the color of the milk that's produced by a purple cow The first thing to do is to find the cow

Philips May I just say parenthetically, for Dr Mudge's benefit that the water buffalo in Egypt, at the time of year when *it is in* heat, gets a purplish tinge

Ussing Of course, there do exist substances in *which the molecular* lattice is so tight that inclosed ions cannot get out Such ions of course would not exchange at all It might be that mitochondria enclose potassium in exactly such a lattice

Mudge I should like to ask Dr Steinbach what data he thinks would be required to demonstrate a potassium pump? *What is the* biological definition of such a mechanism?

Steinbach The requirements at present are essentially thermodynamic, and they are essentially as stated by Dr Ussing In the case of muscle and nerve they are very simple, and easily verifiable

on a quantitative basis Given a certain potential distribution, and given a certain chemical gradient at zero in an equilibrium condition, a "steady-state," then there is no pump, or at least one is not required If the potential difference and chemical gradient are not equal to zero, then a pump is present If you took a microelectrode and stuck it into a liver cell, and found it was highly negative on the inside and positive outside, then a potassium pump is, shall we say, unnecessary, unless there are other reasons to look for it

Berliner If a specific combination of potassium is required to get it in, whether it is with or against the electrochemical gradient, would you call that active transport or not?

Ussing I would not Rosenberg and Wilbrandt (9) have just written a paper discussing the transport of glucose in red cells They point out that certainly the exchange of glucose in these cells depends on some chemical binding But, on the other hand, no net work is involved, and I think that one should only talk about active transport when work is performed by the cell Mere binding to a carrier does not by itself mean active transport

Mudge So, in order to have a pump by these definitions you have to have an energy requirement, you have to put in work In the glucose carrier complex why is there no work done? What is formed?

Taggart I should like to know what kind of chemical reaction is involved

Ussing I really don't know I am just quoting Rosenberg and Wilbrandt The glucose complexes with something which makes it soluble in the membrane and allows it to pass through Then it dissociates off on the other side No work has been performed because the carrier is still the same On the other hand, the carrier may change its chemical composition

Lotspeich Is it like an anion exchange mechanism?

Ussing Exactly But only if the carrier can move spatially, so that it exchanges one time at one boundary, and another time with the other boundary

Berliner But you can conceive of a requirement for a chemical change in the carrier and still transport only with the electro-[illegible]

work in lifting a weight over a wall and down the same level but you have to put work into it to get it high enough to put it over

Steinbach In the one case, you can't measure it

Taggart That is exactly the point Somehow, we always come back to Shannon's famous experiments with the toadfish You will recall that he was studying phenol red transport in a situation where the level of phenol red was higher in the plasma than in the urine In other words, the dye appeared to be running downhill However, the maximal rate of transport remained at its normal value One must conclude that every molecule of dye getting across the tubular epithelium was still actively transported by an energy consuming process Yet, if one approached this situation thermodynamically, it would have to be said that it was a "costless" process

Ussing It might be costless

Taggart It might be, but in this case we know it was not What I wish to emphasize is that the purely thermodynamic approach may be very misleading

Visscher Dr Mudge, have you made any observations of isotopic exchange rate in slices taken out of an intact kidney, or a kidney with a normal blood supply to see whether any of these rate phenomena that you observed in slices can be reproduced under somewhat more normal circumstances?

Mudge Do you mean correlate this with some *in vivo* work?

Visscher Yes

Mudge No we have made no correlations with *in vitro* studies However, our observations are quite similar to the published reports on the equilibration of K^{42} in the kidneys of normal animals

Visscher How long does it take before the specific activity ratio between blood and kidney substance reaches 95, or some such magnitude?

Mudge It is a matter of an hour, I think, in rats

REFERENCES

1 USSING H H Transport of ions across cellular membranes *Physiol Rev* 29, 127 (1949)

2 DAVIES R E, and GALSTON, A W The roll of carbonic anhydrase in ion transport *II Internat Congres de Biochemie* Paris, 1952 (p 142)

3 CROSS R J, TAGGART, J V, COVO G A, and GREEN, D E Studies on the cyclophorase system VI The coupling of oxidation and phosphorylation *J Biol Chem* 177, 655 (1949)

4 HARRIS E J The exchangeability of the potassium of frog muscle studied in phosphate media *J Physiol* 117, 278 (1952)
HOGEBOOM G H, SCHNEIDER W C and PALLADE, G E Cytochemical studies of mammalian tissues, isolation of intact

mitochondria from rat liver, some biochemical properties of mitochondria and submicroscopic particulate material *J Biol Chem* 172, *619 (1948)*
6 Lehninger, A L Esterification of inorganic phosphate coupled to electron transport between dihydrodiphosphopyridine nucleotide and oxygen *J Biol Chem* 178, *625 (1949)*
7 Sanadi, D R, and Littlefield, J W Role of coenzyme A and DPN in the oxidation of α ketoglutarate *Federation Proc* 11, *280 (1952)*
8 Kaufman, S Soluble α ketoglutaric dehydrogenase from heart muscle and coupled phosphorylation *A Symposium on Phosphorus Metabolism* McElroy, W D, and Glass, B, Editors Baltimore, Johns Hopkins Press, 1951, (Vol I, p 370)
9 Rosenberg, T, and Wilbrandt, W Enzymatic processes in cell membrane penetration *Internat Rev Cytology* 1, *65 (1952)*

ION TRANSPORT ACROSS LIVING MEMBRANES

HANS H USSING
Department of Biological Isotope Research
Zoophysiological Laboratory
The University of Copenhagen

ACTIVE TRANSPORT MAY be defined as a transport taking place as a consequence of work being performed by the cells The distinction between active transport and diffusion is usually not too difficult as long as uncharged molecules are concerned, since a behavior in accord with Fick's law of diffusion at least over a certain concentration region, can be taken as proof of simple diffusion Fick's law does not hold for ions, however One has to take into consideration both the concentration difference and the potential difference across the membrane or cell layer, in question The problem may become even more involved in case the drag force arising from the flow of solvent through the membrane has to be taken into account Thus a general solution of the problem of the behavior of an ion under a given set of conditions is quite difficult and probably not to be solved for the time being

However it has turned out that a considerable simplification is obtained if, instead of the net flow of the ion in question one considers the flux ratio that is, the ratio between the influx and the outflux of the ion (1,2 3) The two flux values can be obtained by tracer experiments One can measure influx in one experiment and outflux in another, or what is in many cases more satisfactory, obtain both flux values by measuring one of them with a tracer, and the difference between them or the net amount passing by chemical analysis

But very often one encounters permeabilities so small that the net transfer cannot be determined chemically with any certainty In such cases it is very advantageous to use the double tracer technique (4) for instance, measuring influx using Sodium 22 and outflux using Sodium 24 The two tracers can be easily determined in the same sample, because they have different half lives and because the soft radiation of Sodium 22 can be cut off nearly

quantitatively with a suitable aluminum absorber In a similar way one can obtain both flux values for many other ions

In so far as one can disregard the drag effect of solvent flowing through pores in the membrane the ratio between influx and outflux of a passive ion is related in a very simple way to properties of the system that are easily accessible to measurement This relationship may be expressed as

$$M_{in}/M_{out} = \frac{C_o}{C} \; e^{zFE/RT} \qquad [4]$$

where M_{in} is influx M_{out} is outflux C_o is the concentration of the ion in the external solution C is the concentration in the internal solution Z is the charge of the ion E is the potential difference across the membrane and F R and T have their usual meanings In other words the only values that one need measure are the concentrations in the two bathing solutions and the electrical potential across the membrane and the membrane structure need not be considered This applies to any ion that diffuses passively By "passive" I mean that it does not combine chemically with any moving particle in the system during its passage Therefore deviation from this relationship will appear whether there is active transport or just a binding to a carrier But among the substances which behave according to this Equation we will find those that are not actively transported

I should like to illustrate the application that can be made of this very simple relationship by a few experiments performed mostly on isolated frog skin Of course introducing the subject of frog skin into a discussion on kidney may require some explanation In the first place the frog skin has in common with the kidney the ability to transport sodium chloride and water unidirectionally Secondly frog skin and kidney have another peculiar property in common namely both respond quite actively to posterior lobe extracts I think that these two things taken together justify a discussion of the transport mechanisms present in the frog skin even though the main interest of this group is in the kidney

If we try to apply Equation 4 to the inorganic ions present in the frog skin it is very easily seen that the sodium ion has to be actively transported If we take for example frog skin in contact with Ringer's solution on both sides there may be a potential across it of say 60 mV (the inside positive) and this would make the ratio between influx and outflux of sodium 1 to 10 in other

words ten times as much sodium should go out as comes in Actually, the experiment yields almost exactly the opposite result i e, ten times as much sodium comes in as goes out The equation does not apply, then, and we have to assume that sodium is being actively transported

It is less easily seen whether or not the chloride ions behave actively In the earlier discussion one of the problems brought up was whether or not one could distinguish between active and passive behavior when an ion species was going "downhill I should like to stress that if a passive ion goes downhill Equation 4 would *have* to apply, whereas it would not apply if the ion were actively transported one way or the other

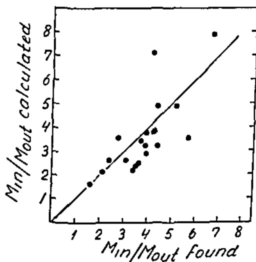


FIGURE 31 Calculated versus found flux ratios (M_{in}/M_{out}) for chloride ions in the isolated surviving frog skin

Figure 31 shows the flux ratios of chloride ions as determined on a number of isolated frog skins (5) The values calculated according to Equation 4 are plotted against the *ratios found* You will see that there is a pretty good correlation between found *and calculated flux ratios*, in other words, the chloride ion seems to behave passively in the isolated frog skin In the experiments presented here there was ten times as much chloride in the inside solution as in the outside solution Thus we had chloride ions moving up a concentration gradient but on the other hand, down the electric potential gradient Offhand one could not have said

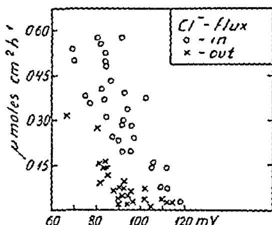


FIGURE 32. Influx (o) and outflux (x) of chloride ions in the isolated surviving frog skin plotted against the spontaneous skin potential. Inside: Ringer; outside: 1/10 Ringer. Reprinted by permission from Koefoed-Johnson, V., Levi, H., and Ussing, H. H.: The mode of passage of chloride ions through the isolated frog skin. *Acta physiol. scandinav.* 25, 157 (1952).

whether the passage was passive or active, but the tracer experiment indicates a passive behavior.

Figure 32 shows the actual flux values found plotted against the spontaneous potential found across the frog skin. It is seen that the higher the flux values the lower the potential difference. This would be in accord with the assumption that the positive sodium ion is transported actively inwards, thus creating an electrical potential difference. The role of the chloride ions in this system would be to bring about a kind of partial short circuit of the electromotive force arising from sodium transport. Thus in cases of high chloride permeability the potential obtained would be low. On the other hand a low chloride permeability, that is a high chloride resistance, would lead to a high potential.

As I say, the data are in accord with this assumption. On the other hand this does not prove at all that the electric potential across the frog skin is actually due to the sodium transport. There might perhaps be other processes going on which contribute to the electric potential. As a matter of fact the whole potential difference might be brought about by something entirely different from the sodium transport. We therefore tried to find some way of demonstrating that the sodium transport was really responsible for the electricity production of the frog skin. It turned out that it could be done by using the short circuiting technique (6).

words, ten times as much sodium should go out as comes in Actually, the experiment yields almost exactly the opposite result i e, ten times as much sodium comes in as goes out The equation does not apply, then, and we have to assume that sodium is being actively transported

It is less easily seen whether or not the chloride ions behave actively In the earlier discussion, one of the problems brought up was whether or not one could distinguish between active and passive behavior when an ion species was going "downhill" I should like to stress that if a passive ion goes downhill Equation 4 would *have* to apply, whereas it would not apply if the ion were actively transported one way or the other

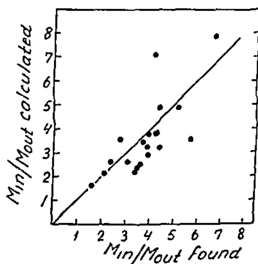


FIGURE 31 Calculated versus found flux ratios (M_{in}/M_{out}) for chloride ions in the isolated surviving frog skin

Figure 31 shows the flux ratios of chloride ions as determined on a number of isolated frog skins (5) The values calculated according to Equation 4 are plotted against the ratios found You will see that there is a pretty good correlation between found and calculated flux ratios, in other words, the chloride ion seems to behave passively in the isolated frog skin In the experiments presented here there was ten times as much chloride in the inside solution as in the outside solution Thus we had chloride ions moving up a concentration gradient but, on the other hand down the electric potential gradient Offhand one could not have said

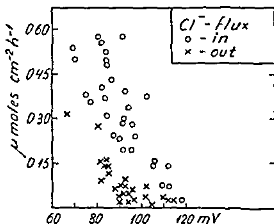


FIGURE 32 Influx (o) and outflux (x) of chloride ions in the isolated surviving frog skin plotted against the spontaneous skin potential Inside Ringer outside 1/10 Ringer Reprinted by permission from Koefoed Johnson V Levi H, and Ussing H H The mode of passage of chloride ions through the isolated frog skin. *Acta physiol scandinav* 25, 157 (1952)

whether the passage was passive or active, but the tracer experiment indicates a passive behavior

Figure 32 shows the actual flux values found, plotted against the spontaneous potential found across the frog skin It is seen that the higher the flux values the lower the potential difference This would be in accord with the assumption that the positive sodium ion is transported actively inwards, thus creating an electrical potential difference The role of the chloride ions in this system would be to bring about a kind of partial short circuit of the electromotive force arising from sodium transport Thus, in cases of high chloride permeability the potential obtained would be low On the other hand a low chloride permeability, that is a high chloride resistance would lead to a high potential

As I say, the data are in accord with this assumption On the other hand this does not prove at all that the electric potential across the frog skin is actually due to the sodium transport There might, perhaps, be other processes going on which contribute to the electric potential As a matter of fact, the whole potential difference might be brought about by something entirely different from the sodium transport We therefore tried to find some way of demonstrating that the sodium transport was really responsible for the electricity production of the frog skin It turned out that it could be done by using the short circuiting technique (6)

TABLE XIV

Comparison Between Na Influx and Short-Circuit Current (Left), and Short-Circuit Current and Na Outflux (Right), in the Short Circuited Frog Skin.*

	Influx mCoulomb cm^{-2} h^{-1}			Outflux mCoulomb cm^{-2} h^{-1}	
	Na	Current		Na	Current
	177	174		9 7	130
	176	162		11 5	139
	248	253		6 2	109
	260	224		8 6	124
	81	74		6 1	136
	106	100		5 6	124
Hind lobe {	168	158	Hind lobe {	8.5	164
	182	155		9 5	164
	57	49		13 6	112
Epinephrine	110	88	Epinephrine	41 0	126
5% CO_2 + 95% O_2 < {	4 5	0	5% CO_2 + 95% O_2 < {	5.5	0
	3 8	0		6 1	0
Air < {	165	150	Air < {	8 3	161
	173	136		15 5	158

*Flux values determined with Na^{24}

with posterior lobe, the short-circuit current, and the sodium flux, are very nearly equal The influx of sodium is slightly higher than the current, but this is made up for by the outflux of sodium Within the accuracy of the measurements, the sodium current is equal to the total electric current created

When a mixture of 5 per cent carbon dioxide, and 95 per cent oxygen, is used in the gas for mixing the solutions, the electric current drops to zero, and the sodium flux drops to a very low value (Table XIV) Indeed it is almost equal to the outflux, so that the net sodium current and the electric current are both nil However, when air is again used for mixing (Table XIV) the electric current returns, and so does the transport of sodium ions This phenomenon is probably attributable to a pH effect in the

interior of the cell If, instead of ordinary Ringer's solution we use a Ringer's high in bicarbonate there is no blocking of sodium transport with five per cent carbon dioxide

We shall now return to the effect of epinephrine (7) The initial experiments (Table XIV) showed that the sodium outflux was very high during epinephrine stimulation As long as influx and outflux were determined in parallel experiments, it was not at all certain whether or not the sodium and the total electric current were equal

TABLE XV

Double Labeling Experiments With Na^{22} (Influx) and Na^{24} (Outflux) on the Short Circuited Surviving Frog Skin *

	In	Out	ΔNa	Current
	μAmp			
Control	233	19 4	213 6	195
Epinephrine	208	110	98	156
Control	292	6 9	285 1	276
Epinephrine	376	93	283	356
Control	166	12 1	154	150
Epinephrine	225	79 4	145 6	212
Control	235	7	228	231
Epinephrine	200	70	130	231

*Control periods show agreement between net Na flux (ΔNa) and electric current Epinephrine periods show additional source of electric current

In the experiments depicted in Table XV, however, sodium influx and outflux were determined simultaneously using Sodium 24 for outflux and Sodium 22 for influx Let us first consider sodium influx and outflux in the control periods Sodium current and net electric current are quite close together But during epinephrine stimulation it turns out to be quite different In all cases the sodium net flux is definitely smaller than the electric current We then looked for some other ion that could be responsible for this discrepancy and it turned out that during epinephrine stimulation

there is an active transport of chloride ions outwards. So far as the electric current is concerned, the effect of a chloride ion passing outward, and a positive ion passing inward, is the same. Thus the two types of current (the chloride current and the sodium current) add up to approximately the total electric current.

We believe that this chloride transport originates in the skin glands rather than in the ordinary epithelial cells, which presumably transport sodium. With epinephrine stimulation there is a very pronounced foaming of the solution, indicating secretion of mucus from the skin glands. It is known that the skin glands are stimulated by epinephrine. But this effect of epinephrine stimulation seems rather unique. We have studied the effects of a large number of drugs and hormones, but no other substance tested could bring about this discrepancy between sodium transport and electric current.

Pitts: How is the chloride secreted?

Ussing: We are playing a trick on the skin by short circuiting it. Ordinarily, of course, the chloride ion would be followed by some cation, but since we short-circuit the skin, the chloride ion can move alone; we supply the counter ions through the electric circuit.

Visscher: Does it secrete hydrochloric acid?

Ussing: I don't think so. I rather think it secretes potassium chloride. We have tried to take samples of the secretion and analyze them for cations and there is an appreciable amount of potassium secretion. There may also be some acid. I don't know.

Table XVI shows a few examples of the effects of drugs (8) on the sodium transport and the oxygen consumption of the short circuited frog skin. It is seen that dinitrophenol and p nitrophenol inhibit sodium transport and stimulate oxygen consumption.

Taggart: What happens with *p* nitrophenol at 5×10^{-5} M?

Ussing: I don't remember. There was an inhibition with sulfanilamide but the molar concentration was quite high, so it may not have anything to do with carbonic anhydrase.

Mudge: From the studies on the kidney, concentrations of sulfonamides high enough to block electrolyte transport decreased oxygen consumption. Is that true also of frog skin?

Ussing: It had no effect on the oxygen consumption of the frog skin. There are two other sulfonamides which were tested. One had no effect on the oxygen consumption, one stimulated it somewhat, and one inhibited it, but all three inhibited the sodium transport. I have recorded the instances (Table XVI) where there was between 25 and 75 per cent inhibition. Quinone inhibits the

TABLE XVI

Effects of Some Drugs on Na Transport and O_2 Consumption of Short Circuited Frog Skin (Fuhrman)
i = inhibition; s = stimulation

	Concentration of Drug* M/l	Effect on Na Transport	Effect on O_2-Consumption
Dinitrophenol	5×10^{-5}	i	s
p nitrophenol	2×10^{-4}	i	s
Sulfanilamide	$2 \times 10^{-}$[illegible]	i	none
p toluene sulfonamide	2×10^{-2}	i	s
Prontosil red	$1 \times 10^{-}$[illegible]	i	i
Quinone	1×10^{-5}	i	none

*Concentration of inhibitor to give necessary 25-75% inhibition of Na transport

sodium transport and has no effect on the oxygen consumption I don't know how to explain it but perhaps someone has a suggestion as to why quinone does that

Before discussing the next Table I should explain its theoretical basis The flux ratio of Equation 4 (M_{in}/M_{out}) is the ratio between two reaction rates if the penetration through the membrane is considered as a chemical process The free energy change ΔF is related to the reaction rate as follows

$$-\Delta F = RT \ln \frac{k_1}{k_2} \qquad [5]$$

where k_1 and k_2 are the rate constants Since the concentrations are the same on both sides of the skin we can substitute rates for rate constants and we have

$$-\Delta F = RT \ln \frac{M_{in}}{M_{out}} \qquad [6]$$

If this consideration is taken to be valid the logarithm of the flux ratio really has the dimensions of a potential and we can calculate this potential in terms of millivolts The difference between M_{in} and M_{out} has the dimensions of electric current strength

We then obtain the partial resistance to sodium by Ohm's law expressed by the equation

$$R_{Na} = \frac{\frac{RT}{zF} \ln \frac{M_{in}}{M_{out}}}{M_{in} - M_{out}} \qquad [7]$$

TABLE XVII

Effect of Different Agents on Active Transport Potential for Na (E_{Na}) and Resistance to Na Ions (R_{Na}) of the Short Circuited Frog Skin

	Concentration of Agent	E_{Na} (mV)	R_{Na} (Ohms/cm²)	Authority
Dinitrophenol	5×10^{-5} M	down	up	Fuhrman (In Press)
Sulfanilamide	2×10^{-2} M	down	up	Fuhrman (In Press)
p toluene sulfonamide	2×10^{-2} M	down	up	Fuhrman (In Press)
Quinone	1×10^{-5} M	down	up	Fuhrman (In Press)
CO_2	5% in air	down	up	Ussing & Zerahn
Tetraethylpyrophosphate (TEPP) (inside solution)	4×10^{-3} M	down	up	Kirschner (Unpublished)
Mersalyl (a mercurial)	1×10^{-4} M	down	up, then down	Linderholm
Aminophylline	0 5%	unaffected	down	Linderholm
Neurohypophyseal extract	50 units/1	unaffected	down	Ussing & Zerahn
Atropine (Rana esc only) (outside solution)	5×10^{-4} M	up	down	Kirschner (Unpublished)
Epinephrine (inside solution)	1×10^{-5} M	down	down	Ussing & Zerahn

Table XVII gives a few examples of such treatment of our data In the presence of dinitrophenol, the electromotive force of the sodium transport mechanism (E_{Na}) goes down, whereas the resistance to sodium ions (R_{Na}) goes up Similarly with sulfonamides, the electromotive force goes down and the resistance up With quinone we have a similar effect Carbon dioxide, that is five per cent carbon dioxide in air or oxygen, does the same, as do tetraethylpyrophosphate, and other inhibitors of acetylcholine esterase

9). They all lower the electromotive force and increase the internal resistance of the "sodium battery" Mersalyl, a mercurial, lowers the electromotive force, but first increases the sodium resistance and then makes it drop (10) Aminophylline does not affect the electromotive force, but lowers the resistance to sodium, with the result that the sodium current increases A similar effect is obtained with neurohypophyseal extracts Perhaps it is pertinent in this context to mention that the flow of water through the skin also increases under the influence of neurohypophyseal extract, apparently as a result of a drop in the resistance to water

Atropine has an effect which is opposite to that of tetraethylpyrophosphate, it increases the electromotive force of the sodium transport mechanism and lowers the resistance to sodium flow (9)

Epinephrine, finally, apart from starting the active chloride transport, has an effect of its own on the "sodium pump," in that it lowers the electromotive force of the sodium transport as well as the resistance to sodium Therefore, during epinephrine stimulation, sometimes you will get more current and sometimes less, depending on whether the drop in electromotive force, or the drop in resistance dominates the picture

TABLE XVIII

Effect of Neurohypophyseal Extract on Electromotive Force, E_{Na}, and Internal Resistance, R_{Na}, of Transporting Mechanism of Frog Skin

	E_{Na}, mV	R_{Na} Ohms/cm²
Control	79 2	2300
Neurohypophyseal Extract	75 9	1660
Control	98 2	4590
Neurohypophyseal Extract	91 6	2640

Table XVIII shows the electromotive force and the partial sodium resistance in a control period, after addition to the inner compartment of neurohypophyseal extract (2) It is seen that E_{Na} remains very much the same whereas R_{Na} is definitely decreased under the influence of the hormone Table XIX shows some experiments which Dr Kirschner did with tetraethylpyrophosphate and atropine Under the influence of TEPP, the electromotive force of

TABLE XIX

Effect of Tetraethylpyrophosphate (TEPP) and Atropine on Active Na-Transport of Short-Circuited Skin of Rana Esc*

	E_{Na} mVolts	R_{Na}, Ohms/cm²	Flux µM/hr In	Flux µM/hr Out	I µAmps
I					
C	35	4060	3.24	0 84	61
4 × 10⁻³ Molar TEPP (Inside)					
E	7	(16500)	0 57	0 46	3
II					
C	45	3030	4 93	0 84	104
4 × 10⁻³ Molar TEPP (Inside)					
E	2	(7000)	0 90	0 82	2
I					
C	35	7000	1 86	0 48	35
1 × 10⁻³ Molar Atropine (Outside)					
E	67	2300	8 66	0 60	205
II					
C	41	3800	3 62	0 78	76
9 × 10⁻³ Molar Atropine (Outside)					
E	66	1570	12 16	1 04	297

*Influx and Outflux µM/hr/7 07 cm²
Current µAmp./7 07 cm²
C Control Period
E Experimental Period

the sodium transport goes down, and the passage goes up considerably Atropine has the opposite effect

Naturally, when we observed the results showing the identity between electric current and active sodium transport, we thought *that perhaps we had the final clue to the electric potentials in the* organism Dr Hogan tried to demonstrate the sodium pump in the short circuited gastric mucosa of the frog Table XX shows *his results The figures for the period when the potential was maintained at zero are particularly striking The influx and the outflux of sodium were practically identical* In other words, in the gastric mucosa there is hardly any active sodium transport The *electric*

TABLE XX

Sodium Influx and Outflux at Different Potentials, in the Gastric Mucosa of Frog*

	Potential Difference mV	Influx (Secretory to Nutrient Side)	Outflux (Nutrient to Secretory Side)
(spontaneous)	0	0 52	0 47
	25	0 42	0 81
	30	0 34	0 83
	56	0 25	1 28

*Ringer's solution on both sides

TABLE XXI

Chloride Flux and Short Circuit-Current; Gastric Mucosa of Frog*

	Potential Differ[illegible] mV	Influx (S[illegible]	O[illegible]	[illegible]	[illegible] out [illegible] in	Electric Current
spontaneous)	0	4 4	5 8	1 32	1 4	1 7
	30	6 4	7 1	1 11	0 7	0
	56	4 7	4 8	1 02	0 1	—

*Ringer's solution on both sides Microequivalents/cm²/hr

current which can be drawn from a short-circuited gastric mucosa must arise from some other process Table XXI, however, shows some figures from a similar experiment with chloride (11) There is a considerable difference between influx and outflux of chloride And what is more, the electric current drawn from the shorted mucosa on the one hand, and the net amount of chloride transported on the other, are practically identical It should be emphasized that in this experiment there was no pH difference, and no difference with respect to composition between the solutions bathing the two sides of the mucosa In this case obviously there must be an active transport of chloride ions As already mentioned, active chloride transport is also seen in the epinephrine-stimulated frog

skin Just before I left, Dr Barker Jørgensen,* in our laboratory, was able to demonstrate that under certain circumstances in the living frog, in contrast to the isolated skin, there is an active transport of chloride inward through the skin The process seems to be an exchange of chloride for bicarbonate Possibly this mechanism is more sensitive to damage to the skin than is the sodium transport This is very fortunate because it enables us to study the active sodium transport in a relatively "pure" state in the isolated skin In the intact frog there seems to be more than one mechanism at work

Thorn Does that hold for nor epinephrine as well?

Ussing We haven't tried that

Dock Is there a temperature coefficient for this transport mechanism? Does it vary with the temperature?

Ussing I think it does, but we haven't studied it with that particular aim in mind

Dock It should drop to zero close to the freezing point, shouldn t it?

Ussing Yes, it probably would

The abolishing of sodium transport by five per cent carbon dioxide astonished us very much Actually *Rana pipiens* is even more sensitive When I was in California three years ago, I used that species Two per cent carbon dioxide in the gas used for mixing lowered the potential to zero At that time I had no means for measuring the short circuit current, but obviously if there is no potential difference there will be no current

Visscher In your earlier work you had a large number of critical studies of the influence of hydrogen ion concentration differences on the two sides of the membrane I seem to recall that in general you found higher alkalinity on the inside of the frog skin to favor the transport Is that correct?

Ussing That is right

Visscher Are you suggesting now that carbon dioxide is producing a pH, at this concentration, that is incompatible with the movement, and if so how do you think it operates in the normal state where there is certainly some carbon dioxide

Ussing In the normal frog there is quite a high bicarbonate concentration, whereas in Ringer's solution there is very little

Visscher But the cells have buffering capacity

Ussing I can t explain it, it is just what we found Linderholm

---

*Jørgensen C B Article under preparation

(10), in Uppsala, has repeated our experiments and found exactly the same

Visscher One question that bothers me a bit is how one can calculate the necessary resistance of a membrane, which consists of innumerable conductances in parallel

Ussing That is perfectly true What we obtain is just an average resistance and an average electromotive force Quite likely the true electromotive force of the transport mechanism is considerably higher This is just as though one took an electric battery and partially shorted it through a resistance One could then take the whole unit and say, "This unit has a certain electromotive force and a certain internal resistance" But these properties need not all reside in the battery proper In the frog skin there may be such an internal short-circuit of the sodium current Unfortunately we cannot get the true electromotive force, at least not for the time being But, even so, the different drugs that act upon the skin seem to fall into various groups Some affect the electromotive force, some the resistance, and some both

Pitts I believe you observed, in some of your experiments, that hydrogen and sodium ions were transported in opposite directions

Ussing Hydrogen ion is transported, at least I think Huf (12) has found that there is a certain leak of hydrogen ions and potassium ions toward the outside The concentration of K^+ and H^+ in the outside medium increases slightly with time but the leakage of these ions represents such a small fraction of the total current that it does not affect the over all result

Pitts It is not nearly as much as the chloride current in the opposite direction?

Ussing That is right

Berliner Do you think of the sodium transport as an exchange for some other ion, such as hydrogen?

Ussing Yes Possibly the carrier exchanges sodium for, say, hydrogen ions or maybe potassium ions A few years ago Solomon proposed that in the red cell there is not one mechanism transporting sodium and another transporting potassium, but a single mechanism exchanging one ion species against the other But I don't know whether he upholds this theory still

Berliner I believe he does not in his last paper (13)

Visscher How much water is moving with the sodium?

Ussing Very little It is about two cubic millimeters per square centimeter per hour It is just so we can pick it up But I think

that it forms the only connection between a solution that is bathing its semipermeable membrane and a compartment containing the pure solvent The solute is assumed to be nonpenetrating Across the semipermeable membrane there will be diffusion of water in both directions and there will be a net water flow from the pure solvent via the osmometer into the solution compartment But in the narrow stem of the osmometer we are not faced with ordinary diffusion but with a mass flow of solvent If heavy water were added to the solution bathing the semipermeable membrane nothing would get to the other compartment if the linear flow rate in the narrow tube were higher than the diffusion rate of water in the opposite direction Thus a membrane consisting of units like this osmometer might appear to be permeable to heavy water in one direction and not in the other Evidently we have to realize that water permeability means more than one thing

If heavy water dissolved in water as solute is used something related to the ordinary diffusion of any solute may be measured However in the case of osmosis it may sometimes be a mass flow That is something that Jacobs (16) pointed out many years ago that diffusion of water and osmosis cannot really be considered identical Although the model experiment discussed is certainly a gross exaggeration of the situation in real living membranes still when water diffusion is taking place over any distance or through a tube of any kind we will have this deviation from ideal behavior The rate of diffusion is determined by the total area available to diffusion whereas the flow depends also upon the diameter of the pores making up this area

Visscher That is a very interesting idea in connection with the rate of equilibration of heavy water across a membrane consisting of let us say eight or ten layers of cells In these cells there might be a considerable impediment to diffusion and less impediment to mass flow particularly if the diffusion were what one might call pericellular rather than transcellular and if the rapid diffusion had to go through extracellular spaces rather than across the cells

Ussing Exactly

Visscher Of course we ordinarily think about membranes as if there were perfect mixing on both sides and this is a gross over simplification of the real situation That is what you are really pointing out

Ussing Yes Of course that also makes it very difficult to calculate the effect of this flow in narrow tubes If moreover there is return of fluid as you suggested in your comments it is likely to

become a very difficult piece of work to find out whether or not it is so

Visscher Have you interpreted the old Hevesy-Krogh observation satisfactorily from your viewpoint?

Ussing If one assumes that the net water transfer across the skin takes place by flow through narrow pores and not by simple diffusion, the Hevesy-Krogh experiments (17) can be explained quite satisfactorily (14) Your observation that the diffusion of heavy water was often too small compared with the net flow of the solution into and out of the gut, might also have some connection with this

Visscher It could have

Pitts I noticed that in one of your Figures (Figure 32) on the membrane resistance, you had a maximum electromotive force of about 120 millivolts Does that set a limiting gradient, against which a sodium pump can transport sodium, of approximately 200 to 1?

Ussing I think it can do better than that In the experiment shown there was beyond the potential difference of 120 millivolts, a concentration ratio of 1 to 10, the outside medium being 1/10 Ringer's, and the inside Ringer's If we add it up, it means an electrochemical potential difference for sodium of 180 millivolts Dr Kirschner, in our laboratory has been doing experiments where he replaced the main part of the sodium chloride in the outside medium by choline chloride Choline ions do not penetrate, whereas the chloride ion is present at the same concentration on both sides of the skin, and thus does not contribute to the potential It turned out that even the merest trace of sodium in the outside medium made the inside solution positive With no sodium in the outside medium there is a diffusion potential due to sodium ions which makes the inside solution slightly negative But as soon as the trace of sodium is added – less than a tenth of a millimole – the potential reverses and again becomes positive It means that under favorable conditions the electromotive force of the sodium pump is at least 200 millivolts

Lotspeich How does that compare with what you find in nerve? I think Hodgkin and Huxley did something on that

Ussing Yes, but they never got potentials as high as that did they?

Lotspeich I don't think so

Ussing And it wasn't frog skin We may have to deal with more than one transporting membrane in series

that it forms the only connection between a solution that is bathing its semipermeable membrane, and a compartment containing t pure solvent The solute is assumed to be nonpenetrating Acro the semipermeable membrane there will be diffusion of water i both directions, and there will be a net water flow from the pur solvent, via the osmometer, into the solution compartment But in the narrow stem of the osmometer, we are not faced with ordinary diffusion, but with a mass flow of solvent If heavy water were added to the solution, bathing the semipermeable membrane, nothing would get to the other compartment if the linear flow rate in the narrow tube were higher than the diffusion rate of water in the opposite direction Thus, a membrane consisting of units like this osmometer might appear to be permeable to heavy water in one direction, and not in the other Evidently we have to realize that water permeability means more than one thing

If heavy water dissolved in water as solute is used, something related to the ordinary diffusion of any solute may be measured However, in the case of osmosis, it may sometimes be a mass flow That is something that Jacobs (16) pointed out many years ago, that diffusion of water and osmosis cannot really be considered identical Although the model experiment discussed is certainly a gross exaggeration of the situation in real living membranes, still when water diffusion is taking place over any distance, or through a tube of any kind, we will have this deviation from ideal behavior The rate of diffusion is determined by the total area available to diffusion, whereas the flow depends also upon the diameter of the pores making up this area

Visscher That is a very interesting idea in connection with the rate of equilibration of heavy water across a membrane consisting of, let us say, eight or ten layers of cells In these cells there might be a considerable impediment to diffusion, and less impediment to mass flow, particularly if the diffusion were what one might call pericellular rather than transcellular, and if the rapid diffusion had to go through extracellular spaces rather than across the cells

Ussing Exactly

Visscher Of course we ordinarily think about membranes as if there were perfect mixing on both sides, and this is a gross oversimplification of the real situation That is what you are really pointing out

Ussing Yes Of course that also makes it very difficult to calculate the effect of this flow in narrow tubes If, moreover, there is return of fluid, as you suggested in your comments, it is likely to

become a very difficult piece of work to find out whether or not it is so

Visscher Have you interpreted the old Hevesy Krogh observation satisfactorily from your viewpoint?

Ussing If one assumes that the net water transfer across the skin takes place by flow through narrow pores and not by simple diffusion the Hevesy Krogh experiments (17) can be explained quite satisfactorily (14) Your observation that the diffusion of heavy water was often too small compared with the net flow of the solution into and out of the gut might also have some connection with this

Visscher It could have

Pitts I noticed that in one of your Figures (Figure 32) on the membrane resistance you had a maximum electromotive force of about 120 millivolts Does that set a limiting gradient against which a sodium pump can transport sodium of approximately 200 to 1?

Ussing I think it can do better than that In the experiment shown there was beyond the potential difference of 120 millivolts a concentration ratio of 1 to 10 the outside medium being 1/10 Ringer's and the inside Ringer's If we add it up it means an electrochemical potential difference for sodium of 180 millivolts Dr Kirschner in our laboratory has been doing experiments where he replaced the main part of the sodium chloride in the outside medium by choline chloride Choline ions do not penetrate whereas the chloride ion is present at the same concentration on both sides of the skin and thus does not contribute to the potential It turned out that even the merest trace of sodium in the outside medium made the inside solution positive With no sodium in the outside medium there is a diffusion potential due to sodium ions which makes the inside solution slightly negative But as soon as the trace of sodium is added — less than a tenth of a millimole — the potential reverses and again becomes positive It means that under favorable conditions the electromotive force of the sodium pump is at least 200 millivolts

Lotspeich How does that compare with what you find in nerve? I think Hodgkin and Huxley did something on that

Ussing Yes but they never got potentials as high as that did they?

Lotspeich I don't think so

Ussing And it wasn't frog skin We may have to deal with more than one transporting membrane in series

that it forms the only connection between a solution that is bathing its semipermeable membrane, and a compartment containing the pure solvent The solute is assumed to be nonpenetrating Across the semipermeable membrane there will be diffusion of water in both directions, and there will be a net water flow from the pure solvent, via the osmometer, into the solution compartment But in the narrow stem of the osmometer, we are not faced with ordinary diffusion, but with a mass flow of solvent If heavy water were added to the solution, bathing the semipermeable membrane, nothing would get to the other compartment if the linear flow rate in the narrow tube were higher than the diffusion rate of water in the opposite direction Thus, a membrane consisting of units like this osmometer might appear to be permeable to heavy water in one direction, and not in the other Evidently we have to realize that water permeability means more than one thing

If heavy water dissolved in water as solute is used, something related to the ordinary diffusion of any solute may be measured However, in the case of osmosis, it may sometimes be a mass flow That is something that Jacobs (16) pointed out many years ago that diffusion of water and osmosis cannot really be considered identical Although the model experiment discussed is certainly a gross exaggeration of the situation in real living membranes still when water diffusion is taking place over any distance, or through a tube of any kind, we will have this deviation from ideal behavior The rate of diffusion is determined by the total area available to diffusion, whereas the flow depends also upon the diameter of the pores making up this area

Visscher That is a very interesting idea in connection with the rate of equilibration of heavy water across a membrane consisting of, let us say, eight or ten layers of cells In these cells there might be a considerable impediment to diffusion, and less impediment to mass flow, particularly if the diffusion were what one might call pericellular rather than transcellular, and if the rapid diffusion had to go through extracellular spaces rather than across the cells

Ussing Exactly

Visscher Of course we ordinarily think about membranes as if there were perfect mixing on both sides, and this is a gross over simplification of the real situation That is what you are really pointing out

Ussing Yes Of course that also makes it very difficult to calculate the effect of this flow in narrow tubes If, moreover, there is return of fluid as you suggested in your comments, it is likely to

become a very difficult piece of work to find out whether or not it is so

Visscher Have you interpreted the old Hevesy-Krogh observation satisfactorily from your viewpoint?

Ussing If one assumes that the net water transfer across the kin takes place by flow through narrow pores, and not by simple iffusion, the Hevesy Krogh experiments can be explained ite satisfactorily (14) Your observation (17) that the diffusion of avy water was often too small compared with the net flow of the ution into and out of the gut, might also have some connection h this

isscher It could have

itts I noticed that in one of your Figures (Figure 32) on the membrane resistance, you had a maximum electromotive force of about 120 millivolts Does that set a limiting gradient, against which a sodium pump can transport sodium, of approximately 200 to 1?

Ussing I think it can do better than that In the experiment shown there was, beyond the potential difference of 120 millivolts, a concentration ratio of 1 to 10, the outside medium being 1/10 Ringer s, and the inside Ringer's If we add it up it means an electrochemical potential difference for sodium of 180 millivolts Dr Kirschner, in our laboratory, has been doing experiments where he replaced the main part of the sodium chloride in the outside medium by choline chloride Choline ions do not penetrate, whereas the chloride ion is present at the same concentration on both sides of the skin, and thus does not contribute to the potential It turned out that even the merest trace of sodium in the outside medium made the inside solution positive With no sodium in the outside medium, there is a diffusion potential due to sodium ions which makes the inside solution slightly negative But as soon as the trace of sodium is added – less than a tenth of a millimole – the potential reverses and again becomes positive It means that under favorable conditions the electromotive force of the sodium pump is at least 200 millivolts

Lotspeich How does that compare with what you find in nerve? I think Hodgkin and Huxley did something on that

Ussing Yes, but they never got potentials as high as that, did they?

Lotspeich I don't think so

Ussing And it wasn t frog skin We may have to deal ... more than one transporting membrane in series

that it has to be exchanged say for potassium would that make the transport of potassium an active process in spite of the fact that it was with the electrochemical gradient or would it not?

Ussing That is just one of the difficult questions but in a way it would in so far as the potassium is forced to do something it would not have done unless it participated in that particular situation

Visscher By your mathematical definition it would not be active transport would it?

Ussing I think it would because the potassium probably would not behave according to Equation 4

Steinbach It would at low concentrations

Ussing It might yes Of course this Equation is just a guide which can be useful under certain circumstances I know that Keynes (18) in Hodgkin s laboratory used this Equation and has been able to show that potassium behaves passively in the nerve

Lotspeich How does the force of this pump compare with the force of the hydrogen ion secretion pump in the kidney? What sort of gradient can be established there?

Ussing I don t know

Lotspeich Have you any ideas on that Dr Pitts?

Pitts The gradient there is somewhere around 8 to 1 The gradient here would be — what?

Ussing You could make it 1000 with 180 millivolts Wouldn t that make a ratio of 1000?

Visscher That is your theoretical maximum gradient What is the probable maximal gradient before the skin can pick up sodium which you have observed?

Ussing I suppose that with a 1/100 millimolar solution on the outside and 100 millimolar on the inside you still may get a positive sign of the inside solution

Visscher So it is 10 000 to 1?

Ussing It probably would be Well I don t know whether it can be so high but it is close to that

Steinbach If you treated frog skin in the same way that Dr Mudge treated kidney slices that is made slices of it and studied ion uptake what would you find?

Ussing I don t know but there is one odd thing if one equilibrates the skin with radioactive potassium and tries to measure the leak out to one side or the other it turns out that nearly all potassium will leak towards the inside Probably therefore the side of

the epithelium cells that faces outward is relatively permeable to potassium compared with the side that faces inward

Visscher And how about sodium?

Ussing Of course sodium goes in faster That is probably due to the transport

Visscher Have you determined the rate at which isotopic equilibrium occurs as far as the sodium in the frog skin cells is concerned?

Ussing Yes

Visscher With sodium placed on either side?

Ussing Yes A steady state is obtained in a matter of some fifteen minutes There is a certain build up period but after about fifteen minutes the flux remains constant which I should say is an indication that a steady state is obtained

Visscher You mean the flux remains constant as judged by the specific activity of the stuff that goes through?

Ussing Yes

Visscher I meant what is the specific activity of the sodium of the frog skin cells themselves? Have you sacrificed the skin and analyzed for total sodium and radioactivity?

Ussing That is one of the things we are trying to do but it is exceedingly difficult because the transporting cells make up a small fraction of the total volume of the skin One would have to slice it We tried freeze drying the skin immediately after experiments

Visscher Have you tried the stratum germinativum to see what that would show?

Ussing The trouble is that even if one freezes in liquid air I don t trust the sodium ion to stay where it should be

Visscher Why not?

Ussing Well it is very diffusible On the other hand it may stay where it is Anyway if we equilibrate the skin with Ringers containing K^{42} and slice it the slice containing the stratum germinativum contains by far the larger part of the radioactive potassium To me that would indicate that the transporting cells themselves contain perhaps more potassium than sodium But I should like to have more evidence that the cells do contain a high concentration of potassium while they transport sodium

Visscher My question was asked because I think the answer to it would help decide whether or not sodium does pass through the cells in its way across

Ussing Well after all the cells have to take care of it in order to transport it to the other side With no potential difference and

muscle of the frog, rather than with a liver slice, or a kidney slice. Otherwise, the procedure was comparable. We started with the freshly isolated sartorius muscle: roughly, 0.1 molar in intrafibral potassium and .01 molar in intrafibral sodium. The muscle was placed in a solution which was essentially 0.1 molar sodium chloride – actually, it had some phosphate buffer also – at about 2°C, in the icebox for about twenty-four hours. We ended up with a muscle *which had approximately .05 M potassium, and .06 sodium,* inside the fibers. The total base (sum of sodium plus potassium) had not changed, but actually with this treatment about half the potassium was lost and replaced by sodium.

The other ions were not measured. I relied upon the fact that if the sum of the concentrations of sodium and potassium remained fairly constant, and if the weight of the muscle did not change (which it did not), probably the result would be a simple exchange of sodium for potassium. If sodium-rich muscle is now put into Ringer's solution 0.01 N in K at about 22° C for thirty minutes to *an hour, you don't quite get complete recovery of the original* ion balance but you almost get it, so for the sake of simplicity in argument, let us just say you return to the original condition.

The questions now arise: what is being moved, and why? As you go from Stage 1 to Stage 2, the initial loss of potassium and the gain of sodium is a simple proposition. It is a "running down hill" affair. The sodium goes according to its chemical gradient, and the potassium goes according to its chemical gradient. The recovery process (Stage 2) however, indicates that the potassium is moving very much against its chemical gradient, and likewise the sodium *is going against its chemical* gradient. As Dr. Ussing has pointed out, if you take into account the potential difference (I don't know what it is here because I haven't measured it although we are in the process of measuring it) you come to the conclusion that the potassium is just sliding in on an electrochemical gradient, and the sodium, on the other hand, is being actively extruded.

To gain more specific evidence for a sodium *transport,* I went through another process of reasoning. Starting with lower potassium content, and relatively high sodium content (Stage 2), we could assume that there is some mechanism which actively pushes potassium into the cell. If it does – and we already know from a variety of evidence that chloride can also go in and out – then the movement of potassium inward should take place regardless of whether or not there is any sodium present in the fiber. *Potassium*

entrance by an active uptake pump should be independent of the sodium concentration within the cell On the other hand if we say in agreement with Dr Ussing and thermodynamics that the potassium moves freely and that the whole thing is due to an outward extrusion of sodium then the net uptake of potassium in recovery could not take place unless there was a net movement of sodium outward If we stopped the movement of sodium outward we should have no potassium uptake

Starting with our initial condition (Stage 1) instead of putting the muscle in sodium chloride solution I put it in buffered 0 1 molar choline 2° C for twenty four hours and ended up with a muscle which had about the same potassium as the sodium chloride extracted muscle This choline treated muscle did not appreciably change in weight so I assumed choline entered in exchange for *sodium and potassium There was little or no sodium left in the* muscle If the choline rich muscle is now put into a recovery solution and left for a couple of hours there is no change in either sodium or potassium

This choline is choline and it is definitely not sodium It might be doing a large number of things to the living fibers in addition to taking the place of sodium In order to obtain a check on the general question of whether choline causes a pharmacological upset somewhere along the line the experiment was repeated with an equimolar mixture of choline and sodium chloride In essence recovery was somewhat intermediate but there was no indication that the presence of choline in the fibers interfered with sodium transport (and potassium uptake) when sodium was available for transport The results are detailed in Table XXII

Pitts You found in any exchange of sodium and potassium a roughly equivalent amount of sodium entering the fiber and potassium leaving the fiber?

Steinbach Yes The frog isolated sartorius is a nice material from that point of view The total base content seems to be fairly well fixed and that is not true in all probability of some of the whole animal experiments on substitution of dietary sodium for potassium *as I suspect that new base binding groups may very well be created* during prolonged potassium depletion or potassium enrichment But in this frog sartorius system apparently you are dealing with something which is physicochemically quite desirable from the biologist's point of view It seems to stay put

Ussing Does the muscle contract after this treatment with choline?

TABLE XXII

Treatment	Time Min	Na Concentration Muscle	Na Concentration Fibers	Na Concentration ΔFibers	K Concentration Muscle	K Concentration Fibers	K Concentration ΔFibers	Final Relative Weight
A								
Extracted.	0	9	8		33	44		
0 11 *M* Choline	15	38	11	+3	40	49	+5	96
0 01 *M* Na	0	7	5		30	40		
Recovery	30	34	5	0	33	40	0	99
0 12 *M* Na	0	8	7		35	47		
0 01 *M* K	90	40	13	+6	41	51	+4	98
	0	7	5		51	68		
	210	36	8	+3	58	73	+5	97
B								
Extracted	0	63	44		42	56		
0 12 *M* Na	30	15	16	—28	58	74	+18	91
Recovery	0	62	43		40	53		
0 11 Choline	90	8	7	—36	60	76	+23	95
0 01 Na								
0 01 K								
C								
Extracted	0	32	21		34	45		
0 055 Choline	30	24	11	—10	50	62	+17	93
0 065 Na	0	31	20		46	61		
Recovery	90	23	9	—11	57	72	+11	94
0 55 Choline								
0 065 Na								
0 01 K								

[illegible] room temperature (ca 22°C) All solutions extraction and [illegible] [illegible] pH 7 2 ± 0 1 with 0 005 *M* sodium phosphate buffer Muscle [illegible]

Reprinted by permission from Steinbach H B On the sodium and potassium balance of isolated frog muscles *Proc Nat Acad Sc* 38, 451 (1952)

Steinbach: I said "no," but I really didn't check it. In preparing these muscles there are always slight areas of necrosis to be trimmed off prior to analysis. The muscles which have been extracted with sodium chloride overnight usually give a little twitch when cut, but the choline-treated muscles rarely do.

Ussing: They do live?

Steinbach: I wouldn't say they are very healthy muscles.

Ussing: But are they really alive?

Steinbach: They are alive in a sense: that is, the choline-treated muscles still completely bar simple equilibration between the sodium outside and the sodium inside. If you kill a muscle by any known means, sodium immediately rushes into that muscle fiber and equilibrates with the external solution. The choline-treated muscles showed absolutely no such equilibration.

Taggart: Have you tried blocking the ultimate uptake of potassium with other inhibitors?

Steinbach: Yes, but this is not as simple as it is in the kidney. The effects of such an agent as iodoacetate upon the whole sartorius muscle are very variable, as compared with the effects on slices or homogenates, so I approached the inhibitor technique with a considerable amount of skepticism. I can select what I think are my best experiments, and they will show that complete blocking of either the oxidative, or the glycolytic, activities will stop this. But then, if you insisted upon seeing all the data, you would find some experiments that showed practically no effect. Temperature, I might add, has a marked influence on this recovery process. The Q_{10} is between 3 and 4, which is rather high.

Mudge: Dr Steinbach and I have been through this before, and I don't see any reason to question the data. My only point is that one must constantly keep in mind the possibility which you mentioned, that is, that low sodium may have some sort of a pharmacologic action on cellular metabolism.

Steinbach: You mean the low sodium, as distinct from the high choline?

Mudge: By lowering sodium you may not be just changing the concentration of one of the reactants in the cation transport mechanism. You may be producing also a lot of other changes, such as directly inhibiting the metabolic reaction that drives electrolyte transport.

Steinbach: Well, let us put it this way. If that is the effect, then it is a greater defect depending upon how much sodium you remove, because I get intermediate values when I use intermediate

Mudge I have made a lot of comparable studies with kidney, replacing sodium with all sorts of things, such as sucrose, lithium mannitol and, after I heard about your results, choline I readily grant that the qO_2 is a fairly crude yardstick, nevertheless, whenever sodium was replaced the qO_2 fell The evidence obtained in studies of kidney indicates that potassium uptake depends on aerobic oxidation, and if the qO_2 is depressed by low sodium I don't see how we can escape the possibility that the effect on electrolyte transport may be due to interference with the metabolic machinery

Steinbach Those experiments are a little different, after all With the muscles there is presumably the replacement of an intracellular cation, sodium or potassium, with another intracellular ion, choline Lithium probably wouldn't work Electrochemically, lithium is very close to sodium

Mudge I was not referring to how the pump works, but simply to effects on the qO_2

Steinbach In the case of nonelectrolytes, the effect on the qO_2 might be very marked, because you would be inducing a considerable depletion of total base, presumably, within the cells

It is one thing to argue about what moves sodium or potassium back and forth, and it is another to try to figure out what an ion does once it gets there There, the zoologist is in a much worse state than he is on the question of ion transport We just do not know With respect to the muscle, the actomyosin, for example, doesn't care whether it is sodium or potassium that it combines with It is perfectly happy with either ion But some of the transphosphorases, as you know, seem to require potassium

Darrow As I understand it, you have no sodium, and no transport of potassium, in the cell, is that right?

Steinbach Yes

Darrow Well, then, when there is choline in the cells, why doesn't the sodium diffuse into the cells and start the pump And yet, there is no evidence that it does In other words, why should there be a pump if it doesn't get started by sodium diffusing into the cells?

Steinbach I should try, I suppose, to do these experiments under optimal survival conditions because, given time enough, you are quite right the sodium leaking in should be available for choline exchange and then be pumped out But that first leakage phase is extremely slow

Darrow Potassium ought to diffuse in cells, too, should it not?

Steinbach I have other data which tend to show that the rate at which extracellular sodium can be extruded is a function of the rate at which potassium can be supplied from the outside

Thorn Is there any way that you could get around this deterioration? Would there be any chance of making these measurements in an electrical field?

Steinbach I might be able to I am not sure It is a very difficult experiment anyway, for other reasons That is why I haven't pushed it any further

Pitts In the last experiment suppose you used the recovery solution and then put it back in the icebox for twenty-four hours?

Darrow You wouldn't be injuring it much more

Steinbach Oh, you might Isolated frog sartorius in the icebox is continually leaking protein, for example

Lotspeich Could you bubble oxygen through the fluid?

Steinbach I could, but I didn t try it

Dock Did you ever have an opportunity to see whether equilibrium would occur in six hours in the icebox?

Steinbach Yes, it doesn t

Dock It takes twenty four hours to reach equilibrium?

Steinbach The initial change, where the sodium enters in and the potassium leaches out is roughly linear in time

Mudge How far down will it go if you keep it over twenty-four hours?

Steinbach That is another question which I cannot answer precisely If much more potassium than that is removed, then the muscle is destroyed The injury potential goes down, it will no longer move the ions It is, in effect, a dead muscle There is a discontinuity which seems to occur at about an internal sodium potassium ratio of 1

Pitts Is that when it breaks down the bound potassium?

Steinbach That's right, it is when sodium goes into mitochondria

Mudge If you do the icebox experiment with kidney slices, in the first half hour potassium will fall from 60 to 20, but in the next twenty four hours it will go down to only about 16, and after another day it is at about the same level But if you keep them longer you find that by seventy-two hours almost all of the potassium has run out

Steinbach Yes, you get a nice flushing-out curve The comparison between the two is that these muscles are very slow compared with the slices, and the same thing is true of recovery

Mudge I have made a lot of comparable studies with kidney replacing *sodium with all* sorts of things, such as sucrose, lithium mannitol and, after I heard about your results, choline I readily grant that the qO_2 is a fairly crude yardstick, nevertheless, when ever sodium was replaced the qO fell The evidence obtained in studies of kidney indicates that potassium uptake depends on aerobic oxidation, and if the qO_2 is depressed by low sodium I don't see how we can escape the possibility that the effect on electrolyte transport may be due to interference with the metabolic machinery

Steinbach Those experiments are a little different, after all With the muscles there is presumably the replacement of an intracellular cation, sodium or potassium, with another intracellular ion choline Lithium probably wouldn't work Electrochemically, lithium is very close to sodium

Mudge I was not referring to how the pump works, but simply to effects on the qO_2

Steinbach In the case of nonelectrolytes, the effect on the qO_2 might be very marked, because you would be inducing a considerable depletion of total base presumably within the cells

It is one thing to argue about what moves sodium or potassium back and forth, and it is another to try to figure out what an ion does once it gets there There, the zoologist is in a much worse state than he is on the question of ion transport We just do not know With respect to the muscle, the actomyosin, for example doesn't care whether it is sodium or potassium that it combines with It is perfectly happy with either ion But some of the trans phosphorases, as you know, seem to require potassium

Darrow As I understand it, you have no sodium, and no transport of potassium, in the cell, is that right?

Steinbach Yes

Darrow Well then when there is choline in the cells, why doesn t the sodium diffuse into the cells and start the pump And yet, there is no evidence that it does In other words, why should there be a pump if it doesn't get started by sodium diffusing into the cells?

Steinbach I should try, I suppose, to do these experiments under optimal survival conditions because, given time enough you are quite right the sodium leaking in should be available for choline exchange and then be pumped out But that first leakage phase is extremely slow

Darrow Potassium ought to diffuse in cells, too should it not?

Steinbach I have other data which tend to show that the rate at which extracellular sodium can be extruded is a function of the rate at which potassium can be supplied from the outside

Thorn Is there any way that you could get around this deterioration? Would there be any chance of making these measurements in an electrical field?

Steinbach I might be able to, I am not sure It is a very difficult experiment anyway, for other reasons That is why I haven't pushed it any further

Pitts In the last experiment, suppose you used the recovery solution and then put it back in the icebox for twenty four hours?

Darrow You wouldn't be injuring it much more

Steinbach Oh, you might Isolated frog sartorius in the icebox is continually leaking protein for example

Lotspeich Could you bubble oxygen through the fluid?

Steinbach I could but I didn t try it

Dock Did you ever have an opportunity to see whether equilibrium would occur in six hours in the icebox?

Steinbach Yes, it doesn't

Dock It takes twenty four hours to reach equilibrium?

Steinbach The initial change where the sodium enters in and the potassium leaches out is roughly linear in time

Mudge How far down will it go if you keep it over twenty four hours?

Steinbach That is another question which I cannot answer precisely If much more potassium than that is removed then the muscle is destroyed The injury potential goes down, it will no longer move the ions It is, in effect a dead muscle There is a discontinuity which seems to occur at about an internal sodium potassium ratio of 1

Pitts Is that when it breaks down the bound potassium?

Steinbach That s right it is when sodium goes into mitochondria

Mudge If you do the icebox experiment with kidney slices, in the first half hour potassium will fall from 60 to 20, but in the next twenty four hours it will go down to only about 16, and after another day it is at about the same level But if you keep them longer you find that by seventy two hours almost all of the potassium has run out

Steinbach Yes, you get a nice flushing out curve The comparison between the two is that these muscles are very slow compared with the slices, and the same thing is true of recovery

17 HEVESY, G VON, HOFER, E, and KROGH, A The permeability of the skin of frogs to water as determined by D_2O and H_2O *Skand arch physiol* 72, *199 (1935)*
18 KEYNES, R D, and LEWIS, P R The resting exchange of radioactive potassium in crab nerve *J Physiol* 113, *73 (1951)*
19 LØVTRUP, S, and PIGON, A Diffusion and active transport of water in the amoeba chaos chaos L *Compt rend Lab Carlsberg, Ser Chim* 28, *1 (1951)*
20 FRANCK, J, and MAYER, J E An osmotic diffusion pump *Arch Biochem* 14, *297 (1947)*

WATER AND ION MOVEMENTS ACROSS INTESTINAL AND RENAL EPITHELIUM

MAURICE B VISSCHER
Department of Physiology
University of Minnesota Medical School

THE INTESTINE DOES interesting things to salt solutions placed within as shown in Figure 35 If sodium chloride and sodium sulfate e placed in a loop of ileum or colon the characteristic result, as

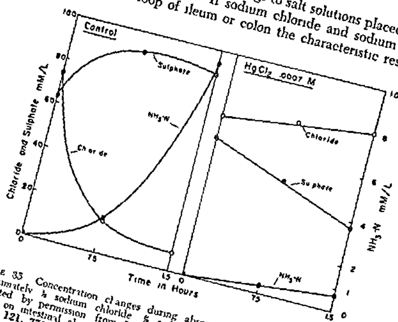


FIGURE 35 Concentration changes during absorption from an isotonic solution approximately ½ sodium chloride ½ sodium sulfate from segments of ileum Reprinted by permission from Ingraham R C and Visscher M B Further studies on intestinal absorption with the performance of osmotic work Am J Physiol 121, 771 (1938)

far as concentrations of sulfate and chloride are concerned is as follows Starting with about 75 mEq of chloride and a little less sulfate in millimols one sees that the former falls and the latter rises This process is greatly influenced by such poisons as mercury, in this instance placed in concentrations of 7/10 000 molar mercuric

17 HEVESY, G VON, HOFER, E, and KROGH, A The permeability of the skin of frogs to water as determined by D_2O and H_2O *Skand arch physiol* 72, *199 (1935)*
18 KEYNES, R D, and LEWIS, P R The resting exchange of radioactive potassium in crab nerve *J Physiol* 113, *73 (1951)*
19 LØVTRUP S, and PIGON, A Diffusion and active transport of water in the amoeba chaos chaos L *Compt rend Lab Carlsberg, Ser Chim* 28, *1 (1951)*
20 FRANCK, J, and MAYER, J E An osmotic diffusion pump *Arch Biochem* 14, *297 (1947)*

can get univalent cation impoverishment in the presence of bi- or tri- or tetravalent cations

Berliner Do you know if there is any pH change in experiments like that?

Visscher Yes, there is However, it will occur at fixed pH

Taggart Are there significant volume changes?

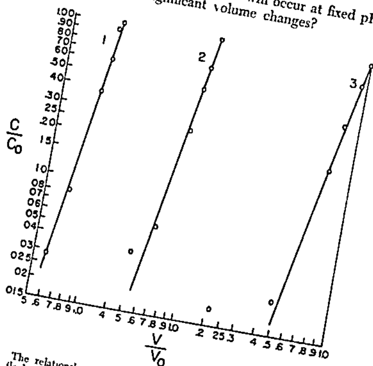


FIGURE 37 The relationship between volume change and chloride concentration changes in ileal segments absorbing isotonic mixtures of sodium chloride and sodium sulfate Reprinted by permission from Ingraham R C Peters H C and Visscher M B On the movement of materials across living membranes against concentration gradients *J Phys Chem* 42, 141 (1938)

Visscher Yes, that is what is shown in Figure 37 the rate of change of concentration is related to the rate of change in volume This Figure presents data from three experiments You will see that if one plots log $\frac{C}{C_o}$ against log $\frac{V}{V_o}$ there is a relationship that is linear over the early period of the experiment This result made us suspicious that there was some important connection between the movement of the ions and the movement of the water and

chloride in the fluid I shall not say much about it because I do not understand the connection, if there is any, myself, but since it might be worth thinking about, I call your attention to the striking difference in ammonia concentration in the two loops This is a regular occurrence with the poisons that are used, whether it has any bearing on mechanism, or whether it is just incidental to a change in permeability to ammonia or ammonium ion, I haven't the slightest idea But with poisons such as fluoride, cyanide, as well as mercuric chloride, this same sort of response is observed Figure 36 shows the effect of arsenite

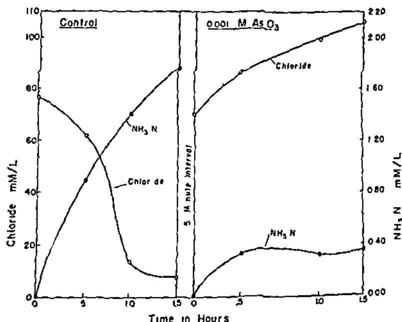


FIGURE 36 The influence of sodium arsenite on chloride impoverishment by the ileum Reprinted by permission from Ingraham R C, and Visscher M B Further studies on intestinal absorption with the performance of osmotic work. *Am J Physiol* 121, 771 (1938)

We called the process chloride impoverishment when we first saw it This kind of chloride impoverishment occurs, not only in the presence of sulfate, but any anion of valence greater than 1 produces the same sort of effect as far as the chloride is concerned Conversely, a comparable thing occurs with regard to cation, particularly sodium, if multivalent cations are present That is, one

can get univalent cation impoverishment in the presence of bi- or tri or tetravalent cations

Berliner Do you know if there is any pH change in experiments like that?

Visscher Yes, there is However, it will occur at fixed pH

Taggart Are there significant volume changes?

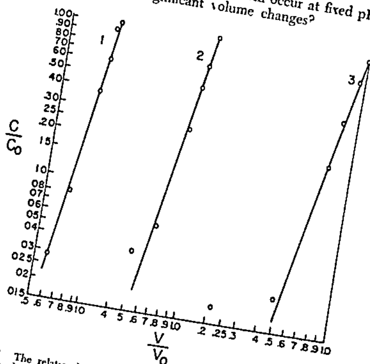


FIGURE 37 The relationship between volume change and chloride concentration changes in ileal segments absorbing isotonic mixtures of sodium chloride and sodium sulfate Reprinted by permission from Ingraham R C Peters H C and Visscher M B On the movement of materials across living membranes against concentration gradients *J Phys Chem* 42, 141 (1938)

Visscher Yes, that is what is shown in Figure 37 the rate of change of concentration is related to the rate of change in volume This Figure presents data from three experiments You will see that if one plots log $\frac{C}{C_o}$ against log $\frac{V}{V_o}$ there is a relationship that is linear over the early period of the experiment This result made us suspicious that there was some important connection between the movement of the ions and the movement of the water, and

much of what we have done in the meantime on this problem has been related to the observation of linearity in the log log relationship This impression was fortified by some other observations

Then we began calculating the clearance, using water labeled with deuterium oxide, and what I should like to call attention to, in Figure 38, is that the ratio of clearance rates for water and chloride (cubic centimeters) approaches 1 as the rate of the chloride removal increases

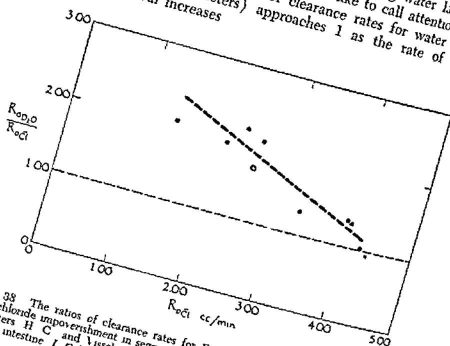


FIGURE 38 The ratios of clearance rates for D_2O and chloride in relation to the rate of chloride impoverishment in segments of dog ileum Reprinted by permission, from Peters H C and Visscher M B On the mechanism of active absorption from the intestine *J Cell & Comp Physiol* 13, 51 (1939)

Steinbach What is the significance of that 1 now? Would that be an isotonic solution?

Visscher It means that for each cubic centimeter of water moved into the blood, the chloride in that volume is also absorbed

Steinbach That means you are taking through a dilute solution?

Visscher That's right

Mudge What does rate of chloride in cubic centimeters mean?

Visscher It is a chloride clearance from the solution in the gut cubic centimeters cleared per minute This is a clearance, and as the clearance increases, the ratio approaches 1 in a large number of observations Again, this fortified our view that there was probably a connection between the water movement and the ion movement

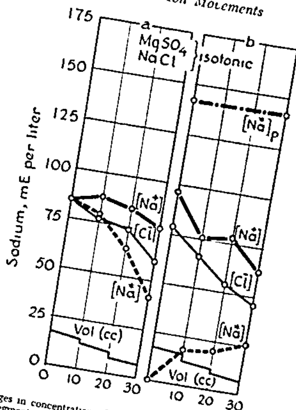


FIGURE 39 Changes in concentration of total sodium chloride and labeled sodium [NA*] from ileal segments (a) With isotopic sodium in the ileal segment (b) With isotopic sodium in the blood Reprinted by permission from Visscher M B Varco, R H Carr C W Dean R B and Erickson D Sodium ion movement between the intestinal lumen and the blood *Am J Physiol* **141**, *498 (1944)*

We began the use of isotopic sodium in the experiments shown in Figure 39, and later work was carried out using chloride and also double-labeling of sodium and water or chloride and water This Figure presents data showing the general sort of result obtained when magnesium sulfate and sodium chloride are placed in an isotonic mixture in a segment of ileum

These experiments were performed with surgically prepared segments of intestine, and the experiments were done on unanesthetized animals trained to lie quietly on a table while observations were being made for as long as thirty minutes On the left are the results of an experiment in which labeled isotopic sodium was placed in the intestine, with the magnesium sulfate sodium chloride solution

You will see that over this period of time there was a small decline in sodium concentration which moved away from an equilibrium concentration Both magnesium and sulfate ions were present Uni univalent salt impoverishment took place and chloride absorption occurred against a concentration gradient It may also be noted that the labeled sodium ion concentration declined to about half of its starting value

We took samples of intestinal fluid hence these breaks in the volume curves at 10 and 20 minutes The volume change aside from these removed samples was assumed to be linear over this period That may be an incorrect assumption but it probably does not introduce any very significant errors The same loop of intestine was studied on another day in an experiment (on the right in Figure 39) in which radio sodium was placed in the blood and the entrance of labeled sodium over thirty minutes was noted The fall in absolute sodium concentration and the fall in absolute chloride concentration are also seen

I wish to point out the difference between jejunum ileum and colon with respect to the slope of the disappearance curve for labeled sodium from those three segments at the level of the bowel as seen in Figure 40 There is a gradient of values for rates the greatest rate being at the oral and the slowest absolute rate at the aboral end of the gut This is not surprising perhaps

Pitts Are these corrected for surface area?

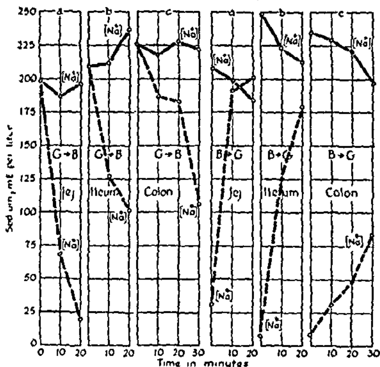
Visscher We used approximately the same length of loop and the same volume

Wallace When you say aboral do you mean the large bowel or terminal end?

Visscher We mean large bowel This is the colon We used a large proportion of the colon to make these loops there wasn t very much left

The first three panels in Figure 40 show measurements of the movement rate of labeled material from gut to blood The right three panels show the movement rate from blood to gut placing the labeled isotope in the blood It will be noted that an aboral gradient for absolute flux rate holds in both cases

Calculations may be made from data such as I have shown you according to rather complex mathematical procedures which will be found in our publications I think the calculation procedure is quite valid it takes into account the back movement from the blood or vice versa when there is diffusion or movement from one compartment to the other and isotopic tracer concentrations



[illegible] isotonic sodium sulfate solu-

ion movement between the intestinal lumen and the blood *Am. J. Physiol.* 141, 438 (1944)

rise in the compartment into which the movement is occurring I think we have taken into account all the major sources of error in the calculation, and have calculated the flux per unit of time from standard solutions of the electrolytes, to which I have referred

In addition to the assumptions that are necessary when one calculates flux from isoptic tracer data, we assumed that the rate of flux (R_{out}) out of the intestine, plus the rate in (R_{in}), taking account of the sign which you can call a minus sign, if you wish, equals the net rate of flux, that is

$$R_{out} - R_{in} = R_{net} \quad [8]$$

Now, this is elementary If one actually measures total movement in two directions, the difference must be the net movement and therefore if one measures any two quantities, one can calculate the other by simple addition or subtraction

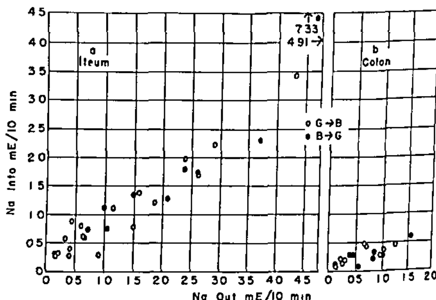


FIGURE 41 The relationship between the absolute rates of movement of sod um ... ed dog prepared ... M B *et al* *Am J Physiol*

In Figure 41 we have plotted on the abscissa values calculated for the flux rate of sodium out of the gut in milliequivalents per 10 minutes against the comparable flux rate for sodium into the gut The open circles are the cases in which the measured tracer movement was from gut to blood That means we put the tracer in the gut The dots are the cases where we measured the movement the absolute flux from blood to gut We also measured the net

One point I want to make, is that the circles and dots fall in the same general region There is no systematic deviation We take this to mean that there is some reliability to the method of measurement But that is not the real point of showing the Figure The real point is the 45° slope The net is measured by the deviation from that slope You will note that in the case of the ileum the deviation is quite different from that of the colon and I would

suggest that it be considered in connection with structures which handle these ions more specifically than the gut does If one made a comparable study, under appropriate circumstances with parts of the renal tubule, one would find even greater deviations from the 45° axis, or the 45° slope I do not know just how one is going to measure this very satisfactorily in the case of the renal tubule, but I think it is something we should be thinking a little about at least

To recapitulate the absolute rates of flux across the epithelium of the colon are lower than in the ileum which again are lower than in the case of the jejunum or duodenum But the relative difference, and the ratio between the rates in and out become greater as the aboral end of the gut is approached, and I would suggest that this is a measure that we ought to try to use in other situations

In some of these experiments we have also been measuring flux rates of water in the two directions by similar means Except for a very few experiments the actual measurements have been from gut to blood because in the days when we were first doing these experiments and were most interested in them deuterium was not easy to obtain and we did not like the idea of putting 1 per cent in the whole of the body water using a 20 kg dog Figure 42 shows some of the rates of water movement observed when one places solutions containing various concentrations of sodium chloride in the intestine The concentrations on the abscissa are the mean values over the time of absorption which was ten minutes If you place an anisotonic solution of sodium chloride in the intestine and let it stay there for ten minutes you will not have the same salt concentration at the end as you had in the beginning Therefore, in order to make these data meaningful we took the arithmetic mean between the original and the final concentration

As Dr Ussing has said there is a real question as to whether the deuterium oxide disappearance can be used appropriately as a measure of total directional movement of water in an osmotic system Krogh and Hevesy had shown some discrepancies about the time we were doing these experiments and we were quite worried about it but we ourselves did some experiments with simple osmometers and without capillary tubes We did not find any discrepancies to worry about However it may still be that the volume of water calculated to have moved is too low I am not sure I do not know how one would calculate the magnitude of the error in reality How would you do it Dr Ussing?

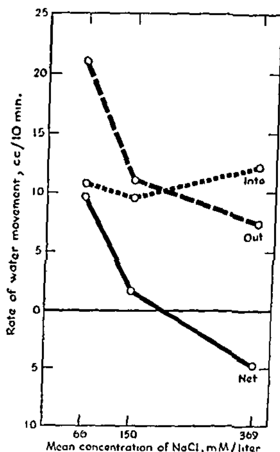


FIGURE 42 Rates of water movement between the lumen of ileal segments and blood in relation to concentration of NaCl in the gut as indicated along the absci The rate of water movement out was calculated from the rate of disappearance deuterium the net movement was measured by the volume change and the i of movement into the gut was calculated as indicated in the text Reprinted permission from Visscher M B Fetcher E S Jr Carr C W Gregor H Bushey M S and Barker D E Isotopic tracer studies on the movement of wa and ions between intestinal lumen and blood *Am J Physiol* 142, 550 (1944)

Ussing We have planned to do some experiments like the: but they are just in the making We should like to find out som thing about the drag force that results when water is osmotical sucked through a membrane Using molecules of graded size, w hope to determine the relationship between pore size and dra force on molecules of known size Then we shall try to use th same setup in living membranes

Visscher That is where you are going to run into trouble

Ussing Yes, exactly, because there may be more than one pore size involved But I hope when the experiments are done, we shall know more about it

Visscher The net water movement is measured as a volume change, and therefore the rates of water movement *into* the gut, as calculated here, are going to be subject to any error that is involved in the calculation of the movements *out* If one assumes that no significant error does enter, then the interpretation of these data would be as follows The rate of water movement into the intestine is uninfluenced by the osmotic activity of the fluid in the intestine That is a perfectly straightforward conclusion which is thermodynamically correct for a diffusion situation It would be very strange if one found anything different on that score because it is the activity of the water on one side of a membrane which will determine the rate at which that water will move to the other side It is the activity on the other side which will determine the rate at which it will move from side 2 to side 1 and it is of course, the difference in the activity that determines the net change It may be that this value, or all of these absolute flux values are too low If they are it would mean that the values for flux into the gut would be moved up on the ordinate but I should be very much surprised if the flux into the intestine were not independent of gut fluid concentration We should then have to find some other factor to account for it, shouldn't we Dr Ussing?

Ussing Certainly After all there are glands in the gut so a certain amount of secretion must be expected

Visscher Yes, that is true and perhaps I should confess right now that in all these experiments we have assumed that whatever glandular activity there is is constant *Otherwise we couldn t really* make our calculations because there is no good way of measuring glandular activity

Figure 43 gets to the next step in the story namely, what can be done if the flux for water and a solute are known Here in Figure 43 we have calculated, the *apparent* or virtual concentration and I stress this, to make it evident that we are not necessarily assuming that this is an actual concentration The apparent concentration of chloride in the water moved out of the intestine is seen to increase with increasing concentration in the lumen That perhaps, is not surprising except for the fact that the apparent concentration of chloride in the water moved into the intestine is uninfluenced by the concentration over the range studied

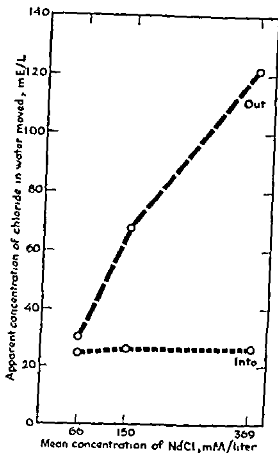


FIGURE 43 Apparent concentration of chloride ion in the water moving between gut and the blood in relation to the gut fluid salt concentration Reprinted by permission from Visscher M B *et al* Isotopic tracer studies on the movement of water and ions between intestinal lumen and blood *Am J Physiol.* 142, 550 (1944)

Darrow If you altered the concentration of sodium in the serum, do you think you would get the same concentration?

Visscher I don't know We have not done it I would doubt that we would have the same concentration I think it is related to the constancy of concentration of chloride in the body fluids

Wallace Haven't you done some work during adrenal insufficiency that might provide the answer?

Visscher We have never carried out tracer studies with adrenal insufficiency only the net studies As a matter of fact, we hope very shortly to do studies with tracers in animals with adrenal insufficiency

Steinbach I don't quite see how you could expect any other result, considering the size of the pools from which movement takes place I can't figure out why you are surprised about it

Visscher I am not really surprised but I think it gives a picture of the movement of water containing electrolyte both out of, and into, the gut, and the difference in concentration of the electrolyte in these two instances is the thing that is responsible for what we call net absorption This is a plausible interpretation of our data and that is all I am suggesting it to be I am sure there are many other possible interpretations and I wouldn't be happy if no one insisted that there were some equally plausible alternatives

Berliner Couldn't the movement of both the water and the chloride be proportional to their concentrations, or more precisely to their activities, since neither the concentration of chloride outside, nor the activity of water outside change appreciably? The movement would then remain more or less in proportion to the fixed ratio outside The reverse would not be true for movement out, since the same fixed ratio does not obtain inside the flux of each would be independent of the other

Visscher Here we are dealing with sodium chloride solutions that are hypo, iso and hypertonic It becomes a little difficult when you take the case of the sodium chloride sodium sulfate mixtures, in which the chloride is moving from a very low concentration in the gut to a much higher concentration in the blood I don't think it would fit this case

Berliner Well, I don't mean to imply that transport is simply diffusion, except in this particular circumstance

Visscher I agree with you that in this instance things proceed in that direction

I should now like to introduce another complication derived from observations made in collaboration with Dr R R Roepke on the osmotic activity of the intestinal contents and blood during the course of absorption of various solutions hyper hypo, and isotonic Figure 44 shows the results from some experiments in which sodium chloride sodium sulfate mixtures were placed in ileal loops of anesthetized dogs, plotted along with the changes observed in chloride concentration You notice that we started with about 75 mEq per liter, and we ended at 75 minutes with 3 to 5 mEq of chloride The osmotic activity (O A) of the plasma was not absolutely constant over this time but it did not change very greatly

In osmolar equivalents of sodium chloride per kilogram of water

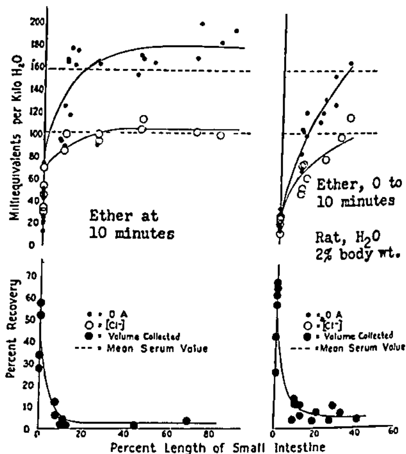


FIGURE 45 Osmotic activities chloride concentrations and volume of fluid recovered ... of the right hand graph the rats were under ether throughout Reprinted with modifications by permission from Follansbee R The osmotic activity of gastro intestinal fluids after water ingestion in the rat *Am J Physiol* **141**, *355 (1945)*

in segments The per cent recovery of fluid in the various segments, the osmotic activity in osmolar equivalents of sodium chloride, and the chloride concentration were measured You will notice that by the time the material had reached or passed the first 20 per cent in length of the small bowel, it had become isotonic In another

experiment the animals were etherized throughout and as you see the fluid remained hypotonic somewhat longer

I show these data because it is a question how mammals which drink so much hypotonic fluid and water get along as well as they do since the intestinal epithelium is surprisingly sensitive to extremely hypotonic solutions Actually if you wash the ileum once with tap water you will not be able to see the phenomenon of univalent salt impoverishment for twenty four hours It is a very fortunate thing that equilibration occurs so rapidly and it is in a sense related to something else I pointed out namely the rapidity with which ions move in both directions at the oral end of the intestine I might also mention that another of my associates Mr J S Lee has found that if as much as 10 per cent of body weight in water is given by stomach tube to rats detectable hemolysis in the blood and hemoglobin in the urine will appear in about 75 per cent of the cases He has shown further that there is a higher concentration of free hemoglobin in the portal vein blood than there is in the rest of the vascular bed indicating I think that one can get osmotic hemolysis from the rapid inflow of water into the oral end of the small intestine

Mudge How much water is required for that?

Visscher Ten per cent of the body weight produces it fairly regularly and five per cent only occasionally

Lotspeich Dr Visscher you mentioned that in the movement of sodium and chloride into the gut glandular activity is assumed to be constant It is not clear in my mind whether you consider the movement of ions into the gut to be characteristic of any membrane or whether they are actually parts of the secretion of succus entericus which certainly is fairly rich in those ions is it not?

Visscher Yes it is

Lotspeich I dont see how you can differentiate the glandular problem or dismiss it quite so quickly

Visscher I would not say that we were dismissing it We are tentatively ignoring it for the purpose of simplification however it may be oversimplification

Lotspeich Looking at it teleologically I do not understand the purpose of the influx of water and ions into the intestine in order to get them out in the end I have never been able to understand that aspect of this problem

Visscher Lets look at it the other way around There is an influx isnt there?

Lotspeich Yes

Visscher And since there is an influx why shouldn t it be used? If you are arguing teleologically I think there is just as good a case for the argument that it should be used since it exists

Oliver The process is correcting an earlier mistake in other words

Visscher It might be I do not know that it is a mistake that is I think it may be absolutely essential to have relatively rapid movements of one thing and another in living systems in order to get mixing if nothing else If those movements can be utilized for concentration work why not do so? I have not used that as a basis for my reasoning but since the problem of teleology has been brought up I see no reason why it should not be used

Dock If you use a membrane like urinary bladder is influx very small or is there a big turnover?

Visscher It is very small The urinary bladder is not impermeable to water by any means but the flux rates are a small percentage of the values found in the gut

Dock It must be very expensive to make a columnar epithelium in order to prevent small ions which make no difference to the body anyway from passing back and forth through it It would seem to me that a single layer of epithelium allowing chloride and water to go back and forth as fast as they wanted to would be the cheapest solution It costs money to make a waterproof jacket like the covering of the bladder

Visscher It probably costs even more money to make ionproof jackets and what seems to be the case in the gut at the upper end of the alimentary tract is that they are not ionproof and that at the lower end there is a certain amount of ionproofness which allows you to obtain the net absorption

Dock It is relative ionproofing isn t it? There is still a large turnover compared with excretion

Visscher Yes it is all relative

Pitts This ratio of concentration of ions and water entering the gastrointestinal tract and remaining constant is really a two phase system — one part diffusion the other part secretion — is it not? Does that indicate a flux of water without ions?

Visscher Perhaps it is all secretion

Pitts Or mixed with a small volume of secretion in which ions are relatively concentrated Is that the way you break it down?

Visscher When we plot our data in a slightly different way we can show that in the cases where the univalent ion impoverishment is most rapid the virtual concentration of electrolytes in the water

moving into the gut is the lowest In other words, when we find very low concentrations, such as a few milliequivalents per liter for chloride in the intestine, and measure the virtual concentration of chloride in the water, we can calculate, from isotopic tracer data on the movement into the intestine at that time, that concentration approaches zero

Pitts Maybe your hypotonic solutions are stimulating secretion, and the less hypotonic or the more nearly isotonic, they are the less secretion is provoked

Visscher It is possible

Ussing But this treatment does assume then, that there is absolutely no water permeability to those cells, that is apart from the active transport of water in both directions

Visscher You mean an extreme application of the theory that I have set forth?

Ussing Yes

Visscher At one end of the extreme it would be required That is correct But I do not think we need to make that assumption Dr Ussing I think it is more complicated than that

Ussing I just wanted to point out that it would be surprising if the cells had *no passive water permeability*

Visscher I am sure they do have some

Ussing But it would throw off the calculation of the amount of salts in the moving water

Visscher Yes We should have to say that the amount of fluid moving into some other stream with salt in it would be smaller

Ussing In other words, the salt concentration would be higher

Visscher That s right

Ussing That brings me back to the question could it be the salt transport which drags along the water?

Visscher It could if you had pure water moving across some other way But it would have to be virtually pure water

Ussing I am not so certain about that The whole problem would have to be reconsidered in the light of the theory of solutions moving through pores The following Equation is approximately valid for any type of flow

$$\ln \frac{M_{in}}{M_{out}} = \ln \frac{a_o}{a_i} + \frac{\Delta_w}{D_w} \int_0^{x_o} \frac{1}{A}\, dx \qquad [9]$$

D_w is the diffusion coefficient for water diffusing in water M_{in} is the influx of water, M_{out} is the outflux, a_o and a_i are the activities of the water in the outside and the inside compartments, respectively, Δ_w is the net flux of water ($M_{in} - M_{out}$), A is the fraction of the total area which is available to flow and diffusion of water molecules, x is the distance from the inside boundary, and x_o is the total thickness of the membrane

This equation describes the interdependency between total water flux and net water flux in the amphibian skin, quite satisfactorily, and thus explains Krogh's old data on the frog skin This is a purely formal description and cannot be used to distinguish between active transport and passive flow But in all cases of passive flow, this or a similar equation should apply

Philips What would happen if you produced acidosis or alkalosis? I am asking this question because of our experience in the treatment of patients with cholera It was rather striking that after the patient was brought back into an acid base equilibrium and rehydrated, he recovered from the disease That was true over quite a period of time Hence, I wonder if it is possible that in acidosis it would take longer and longer, going down the intestine before osmotic equilibrium and isotonicity would be reached

Visscher We have never studied deuterium oxide movement with changes in hydrogen ion concentration, only the univalent salt impoverishment in relation to hydrogen ion concentration

Pitts Dr Wallace has some work which I think is appropriate to the discussion

Wallace As pediatricians we have been interested in trying to find out why so much potassium is lost in diarrhoeal stools A closely allied problem is the hyperchloremic acidosis that occurs in patients when the surgeon implants the ureters in the colon We originally started by studying the changes occurring in the ionic composition of bicarbonate Ringer s when it is placed in the isolated loops of infants undergoing various types of bowel surgery Dr William Schwartz and his associates, at Tufts, have studied what happens to 155 mM sodium chloride solution instilled into isolated loops of the large bowel in dogs Figure 46 shows some of their data on the behavior of chloride and bicarbonate The change in chloride concentration is similar to that shown in some of Dr Visscher's Figures which we saw earlier The concentration of chloride falls, while that of carbon dioxide increases in such a way as to keep the sum of the two approximately constant

Taggart That is bicarbonate?

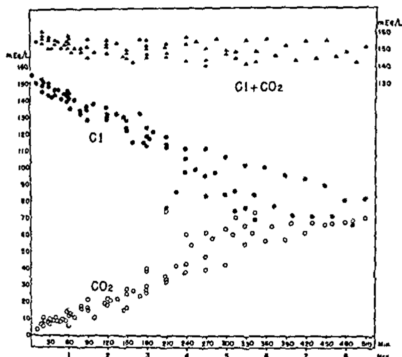


FIGURE [illegible]
carbor [illegible]
isolate [illegible]
better [illegible]
saline [illegible]

Wallace That is total carbon dioxide We have made a few pH measurements that indicated an increase of pH to about 7 5 – 8 0 in some of the experiments with babies I had planned to present some calculations on the bicarbonate concentration and CO_2 tension made from pH and total CO_2 contents of colonic fluid from other experiments of ours However, Dr Visscher tells me that pK CO_2 values for gut fluid may be as low as 5 5 I had used the value for isotonic salt solution This would probably get you into difficulty, wouldn't it, Dr Visscher?

Visscher I think so

Wallace Figure 47 shows the changes that occur in the cations during the process The problem of why stools have such a high potassium concentration may be raised here The Figure does not show it in a very convincing fashion but one can see that as

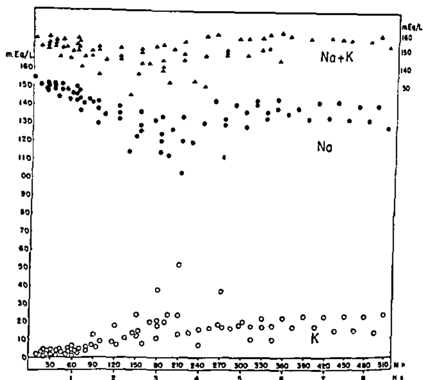


FIGURE 47 Changes occurring with time in the concentration of sodium and potassium after the instillation of 155mM sodium chloride solution into isolated colonic loops of the dog Reprinted by permission from D Agostino A Leadbetter W F and Schwartz W B Alterations in the ionic composition of isotonic saline solution instilled into the colon *J Clin Investigation* 32, 414 (1953)

chloride falls sodium decreases but to a lesser degree, and potas sium increases in concentration The sum of sodium and potassium ends to stay constant I think the observations are of interest because they suggest that as chloride and bicarbonate are ex hanged, potassium tends to take part in the transaction One wonders — and I wanted to draw Dr Ussing out on this — does this ndicate active transport of chloride by the gut? Perhaps this will ome out in a later discussion

Elkinton Dr Wallace, how do you reconcile those two curves with the fact that in the stool you actually have a great deal more potassium than sodium?

Wallace I don't know

Elkinton Here, your curves only approach each other, they do ot actually cross

Wallace We did some experiments in which we put isotonic (155 mM per L) potassium chloride in the gut, and found that the potassium concentration decreased to about 40 mM per liter Similarly, when we put a fluid with no potassium in the gut the concentration approached 40 mM per L and in both instances chloride tended to decrease in concentration

Pitts Sodium rises on that, too

Wallace Yes, the opposite with potassium chloride

Thorn Did you mean hypochloremic or hyperchloremic? The typical syndrome with ureteral implantation in man is elevation of the serum chloride level

Wallace There seems to be an overabsorption of chloride

Visscher I think time is of the essence in all these processes, and we should avoid trapping ourselves in our interpretations Maybe some of mine are subject to that error, I am not sure But along the lines that have been developed by Dr Wallace, I wanted to answer the question that was raised earlier about the pH changes in the intestine – and this is lower ileum – while absorption of the sodium chloride – sodium sulfate was going on In a period of ninety minutes, the initial pH of the fluid was 7 05 and the final pH was 6 34 In this case, chloride fell from 73 3 to 3 7 mEq per liter, and carbon dioxide rose from 0 to 4 8, while at the end the plasma values were 7 18 for pH, 102 mEq for chloride, and for total carbon dioxide 25 mM per liter

Now, this is one experiment among many If one starts out deliberately with a lower pH, say 5 5, one finds that it tends to rise, but not to neutrality Of course, the anions must balance the cations, and if a readily diffusible anion is not present, and there is chloride removal, there will be substitution with an anion that can be produced locally, like bicarbonate In the case of absorption of pure sodium chloride, the total carbon dioxide will be higher Incidentally, while getting absorption in the study of the absorption of autogenous serum, the carbon dioxide value rises inordinately in the serum in the gut, because the bicarbonate is left in, and the chloride is removed

Wallace What happens to carbon dioxide tension?

Visscher I have to plead that the precise values are not in my mind, because we never published them What we actually did in making these measurements, in order to be quite sure of our ground, was to have a two-phase system in the gut We put a gas phase along with the fluid phase, and then waited until equilibrium occurred in the gas phase by measurement of the gases with the

Haldane apparatus We then used the gas phase, with which the fluid had been in equilibrium in the gut, in the segments of the intestine, to flush through our small chamber in the thermoelectric vapor tension apparatus We thought that would help us to know exactly where we were

Pitts Is that the way you got the pK's, too?

Visscher At that time we tried to determine the pK for gut fluid and found that no two samples of gut fluid under these circumstances gave us the same values Again we have never published those data We were frustrated somewhat on that score, but it is a problem which I think would be worthy of further investigation We may get to it sometime

Dr Wallace I think this falls in line with your observations Actually, if an exogenous polyvalent anion is not present and chloride is removed, bicarbonate ion will increase

Berliner One question Do you know what happens to the pH when you have nonabsorbed cation? Does it go up higher than it does when you have, say, sodium chloride?

Visscher Yes

Berliner It is interesting that that is just what happens in the kidney When you have anions which cannot be reabsorbed you get very acid urine and when you have non reabsorbed cation you are likely to get very alkaline urine

Visscher There are several ways of looking at this problem and I think you might be interested in some additional data which we have obtained in our laboratories bearing on intestinal absorption from another viewpoint Two of my collaborators Dr Eugene Grimm and Mr J S Lee, are primarily responsible for the data which I shall discuss next having to do with the distribution between the blood vascular channels and the lymphatic channels in the intestine, of materials that undergo absorption in the intestine We know that the intestine has a very ample structural arrangement for lymph flow, and that certain materials may be absorbed to a fairly large extent through the lymphatics Previously we had not considered the possibility that lymphatic channels might be of some importance in connection with intestinal absorption, and recently, therefore, we felt that it was worth making some studies

Fluid containing deuterium oxide in concentrations of 2 2 or 1 1 per cent was placed into an intestinal segment, and blood was collected from the femoral artery and from the mesenteric veins draining the segment of intestine while lymph was obtained from

TABLE XXIII
The D_2O Concentration of Blood and Lymph During D_2O Water Lavage

Dog	D_2O Concentration (wt %)			
	Lavage Fluid	Mesenteric Vein	Mesenteric Lymph	Femoral Artery
1	2.21	0.15	0.03	0.03
2	1.11	0.07 (2)	0.03 (3)	0.02 (2)
3	1.11	0.03	0.00	0.00
4	1.11	0.10 (3)	0.01 (2)	0.01 (3)
5	1.11	0.10 (2)	0.01	0.01 (2)
6	1.11	0.05 (3)	0.01	0.01 (3)
22	1.10*	0.04 (2)	0.015(2)	0.01
Mean		0.08	0.02	0.01

*In this case D_2O-1/3 Isotonic Krebs solution replaced water as the Lavage Fluid. Numerals in parentheses indicate the number of samples collected if greater than one.

Unpublished data from experiments of J. S. Lee, E. Grim and M. B. Visscher.

all the visible lymphatics in the mesentery. The concentrations of heavy water in blood and lymph are set out in Tables XXIII and XXIV. The D_2O concentration in the mesentery vein blood which comes from the segment is higher than the femoral artery blood D_2O, which of course, is elevated above the normal isotope distribution rates because these experiments go on for some time. In this case there may have been absorption for an hour or two from the loop before these measurements were made. If a fairly large fraction of the blood collected from the veins coming from the given segment of intestine has passed through capillaries close to the intestinal epithelium and if equilibration across a single layer or a couple of layers of cells should be virtually complete in one circuit of the blood, we would expect to find considerably higher figures.

This finding has several possible interpretations. For example it might mean that the mucosal surface is less permeable to water by diffusion than one had supposed and that there is a fixed and

TABLE XXIV

The Effect of Salt Concentration in D_2O-Solution Lavage on the D_2O Concentration of Mesenteric Venous Blood

Dog	Approx D_2O Conc Lavage Fluid	Venous D_2O Concentration (wt %)		
		Isotonic Lavage	1/3 Isotonic Lavage	Isotonic Lavage
13	2	0.06 (3)	0.10 (2)	0.07 (1)
15	2	0.04 (2)	0.05 (3)	0.05 (3)
16	2	0.03 (3)	0.13 (3)	0.08 (3)
21	2	0.03 (2)	0.14 (2)	0.07 (2)
17	2	0.05 (2)	0.09 (2)	——
22	1	0.04 (2)	0.04 (1)	0.04 (1)
Mean		0.04	0.09	0.06

Numerals in parentheses indicate the number of samples collected the D_2O concentration given being the mean value

Unpublished data from experiments of E Grim J S Lee and M B Visscher

controlled rate of water movement, or one might conclude that only about five per cent of all the blood going to a segment of the intestine actually perfuses the intestinal mucosa I personally find the latter interpretation very difficult to accept without more evidence.

Pitts Have you scraped off the villous surface, just to get the very superficial layer of mucosa, to see what its relative deuterium oxide content is?

Visscher The superficial layer of mucosa has a high deuterium oxide content

Pitts You would assume, then, that the interstitial core of each villus is in essential equilibrium with the fluid content of the gut?

Visscher I should think so

What we propose to do is to remove the intestinal mucosa entirely, and repeat the experiments in segments of intestine less mucosa, which, oddly enough, can be done without so much hemorrhage that the segment cannot be used again We have not done that experiment, but it is our next step

It is surprising that the values for D_2O concentration in the lymph approach the arterial concentrations much more closely than they do the venous concentrations. When lymph is collected, let us say five centimeters away from the intestinal mucosa, these are the values obtained. It might be supposed that the lymph being collected was not really formed in the villi and mucosa of the gut, and that the formation of villus lymph is very low under conditions like these, where there is salt solution in the intestine. This *is possible. It is also possible, I think, that a lymphatic which* starts in the villus, may pass through other structures at a distance from the gut lumen, with re-equilibration of water. The submucosa has a capillary network filled with blood which has never approached the mucosal surface and re-equilibration under these circumstances would yield values for lymphatic D_2O close to those for arterial D_2O. I do not know whether or not that is the answer but it is certainly one possible interpretation of the data I have just shown you. I mention it primarily because I think we have to be cautious about making what appear to be the simplest possible interpretations of our data in connection with problems like these. This is certainly not what we expected to find.

Steinbach Isn't the rate of lymph flow very slow?

Visscher Yes, it is.

Steinbach Why isn't the lymph just tending towards equilibrium with this body fluid around the mesentery?

Visscher That is a variant of the idea that I have just given you.

Thorn What is the actual rate of disappearance from the lumen of the bowel?

Visscher There is no net loss over the time of study. I should like to mention the fact that the rate of lymph flow seems to be increased when one puts a hypotonic solution in the intestine as compared with the isotonic solution. I don't want to stress that point too much, because the number of experiments is small. Likewise, with regard to the blood flow there is evidence that there is a slightly *increased blood flow* when hypotonic solutions are in the gut, as compared with isotonic solutions.

I also want to present a few data on transport of potassium and sodium as measured by isotopic tracers. A sample experiment with Potassium 42 is shown in Table XXV (A). It will be seen tha[illegible] except in the case of the hypotonic solution the lymphatic s[illegible] activity is less than the venous. That for the mucosa is less t[illegible] for the lavage fluid, in fact about one-ninth. The muscularis [illegible] a still lower value. In other words the K^{42} was being

TABLE XXV

The Relation of Concentration of Lavage Fluid to the Absorption of Isotopic Potassium and Sodium

(A) Potassium (K^{42})

Origin of *Sample*	Specific Activity (Counts/min/mEq $\times 10^{-3}$)		
	Isotonic Lavage	1/3 Isotonic Lavage	Isotonic Lavage
Lavage Fluid	*8800*	*9500*	*8800*
Lymph	18	295 (2)	40
Mesenteric Venous Blood	58 (2)	95 (2)	65
Femoral Arterial Blood	0	3	1
Intestinal Mucosa			*1000**
Intestinal Muscle			*300**

*At conclusion of experiment

(B) Sodium (Na^{22})

Origin of Sample	Specific Activity (Counts/min/mEq $\times 10^{-3}$)		
	Isotonic Lavage	*1/3 Isotonic* Lavage	*Isotonic* Lavage
Lavage *Fluid*	*1720*	*5420*	0
Lymph	1 5	37 1 (2)	13 9
Mesenteric Venous Blood	5 4 (2)	9 8 (2)	1 9 (2)
Mesenteric *Venous Plasma*	13 6 (2)	25 7 (2)	4 1 (2)
Femoral Arterial Blood	0 8	1 6*	
Femoral Arterial Plasma	2 3	4 1*	

*This sample withdrawn at end of the period of Na^{22} Lavage
Numerals in parentheses indicate the number of samples collected if greater than one, the specific activity given being the mean value

Unpublished data from experiments of E Grim J S Lee, and M B Visscher

from the outer layers even of the mucosa by "arterial" blood which did not come within effective diffusion distances from the gut lumen

Pitts This was 1000 at the time the lymph was 40 and the mesenteric venous blood was 65?

Visscher Yes With regard to venous blood of course potassium in the red cells had not come into equilibrium in this time therefore we might perhaps double these values or a little less than double to obtain a figure that would be the proper value for the plasma potassium You will note again that with regard to the lymph while the isotonic solution is being absorbed it is closer to the femoral arterial blood than it is to the mesenteric venous blood However with the one third isotonic solution if one doubles the value one still finds that the lymph value is higher One would conclude then that there is quite a bit of the labeled potassium in the lymph as collected when a hypotonic solution is being absorbed This one experiment is typical of about four others and we think it is quite reliable

In the case of sodium in a similar experiment one finds as seen in Table XXI (B) a somewhat comparable effect the specific activity in the lymph is closer to the femoral arterial than to the mesenteric venous plasma in the case of the isotonic and the reverse is true in the case of the hypotonic solution The fact that the lymph still contains radioactivity after the fluid in the intestine has been changed so that there is no radioactivity in it I think simply means that we are washing out lymph that was formed during the earlier period It probably represents I think a technical inadequacy Of course there was radioactivity in the venous plasma too Perhaps I had better change that and say that there is radioactivity in the sodium in the wall of the segment of gut and that it is being washed out slowly

Now I should like to present a few data from experiments in our laboratories for which Mr T Hoshiko and Mr R E Swanson are mainly responsible They deal with double perfusion isotopic tracer studies of renal tubule studies in the bullfrog We have measured the movement of the materials across the renal tubule in the frog because of the availability of the renal portal vein as a portal of entry for blood which largely perfuses the capillaries going to the tubules under proper pressure conditions in the renal artery and renal portal vein One can distinguish satisfactorily between the materials appearing in urine by glomerular filtration and the materials that reach the urine via trans tubular transport

TABLE XXV

The Relation of Concentration of Lavage Fluid to the Absorption of Isotopic Potassium and Sodium

(A) Potassium (K^{42})

Origin of Sample	Specific Activity (Counts/min/mEq × 10^{-3})		
	Isotonic Lavage	1/3 Isotonic Lavage	Isotonic Lavage
Lavage Fluid	8800	9500	8800
Lymph	18	295 (2)	40
Mesenteric Venous Blood	58 (2)	95 (2)	65
Femoral Arterial Blood	0	3	1
Intestinal Mucosa			1000*
Intestinal Muscle			300*

*At conclusion of experiment

(B) Sodium (Na^{22})

Origin of Sample	Specific Activity (Counts/min/mEq × 10^{-3})		
	Isotonic Lavage	1/3 Isotonic Lavage	Isotonic Lavage
Lavage Fluid	1720	5420	0
Lymph	1 5	37 1 (2)	13 9
Mesenteric Venous Blood	5 4 (2)	9 8 (2)	1 9 (2)
Mesenteric Venous Plasma	13 6 (2)	25 7 (2)	4 1 (2)
Femoral Arterial Blood	0 8	1 6*	
Femoral Arterial Plasma	2 3	4 1*	

*This sample withdrawn at end of the period of Na^{22} Lavage
Numerals in parentheses indicate the number of samples collected if greater than one, the specific activity given being the mean value

Unpublished data from experiments of E Grim J S Lee, and M B Visscher

from the outer layers even of the mucosa by "arterial" blood which did not come within effective diffusion distances from the gut lumen

Pitts This was 1000 at the time the lymph was 40 and the *mesenteric venous blood was 65?*

Visscher Yes With regard to venous blood of course potassium in the red cells had not come into equilibrium in this time there fore we might perhaps double these values or a little less than double to obtain a figure that would be the proper value for the plasma potassium You will note again that with regard to the lymph while the isotonic solution is being absorbed it is closer to the femoral arterial blood than it is to the mesenteric venous blood However with the one third isotonic solution if one doubles the value one still finds that the lymph value is higher One would conclude then that there is quite a bit of the labeled potassium *in the lymph as collected when a hypotonic solution is being* absorbed This one experiment is typical of about four others and we think it is quite reliable

In the case of sodium in a similar experiment one finds as seen in Table XXV (B) a somewhat comparable effect the specific activity in the lymph is closer to the femoral arterial than to the mesenteric venous plasma in the case of the isotonic and the reve [illegible] the [illegible] has [illegible] simply means that we are washing out lymph that was formed during the earlier period *It probably represents,* I think a tech nical inadequacy Of course there was radioactivity in the venous plasma too Perhaps I had better change that and say that there is radioactivity in the sodium in the wall of the segment of gut and that it is being washed out slowly

Now I should like to present a few data from experiments in *our laboratories for which Mr T Hoshiko and Mr R E Swanson* are mainly responsible They deal with double perfusion isotopic tracer studies of renal tubule studies in the bullfrog We have measured the movement of the materials across the renal tubule in the frog because of the availability of the renal portal vein as a portal of entry for blood which largely perfuses the capillaries going to the tubules under proper pressure conditions in the renal artery and renal portal vein One can distinguish satisfactorily between the materials appearing in urine by glomerular filtration and the materials that reach the urine via trans tubular transport

TABLE XXV

The Relation of Concentration of Lavage Fluid to the Absorption of Isotopic Potassium and Sodium

(A) Potassium (K^{42})

Origin of Sample	Specific Activity (Counts/min/mEq × 10-3)		
	Isotonic Lavage	1/3 Isotonic Lavage	Isotonic Lavage
Lavage Fluid	8800	9500	8800
Lymph	18	295 (2)	40
Mesenteric Venous Blood	58 (2)	95 (2)	65
Femoral Arterial Blood	0	3	1
Intestinal Mucosa			1000*
Intestinal Muscle			300*

*At conclusion of experiment

(B) Sodium (Na^{22})

Origin of Sample	Specific Activity (Counts/min/mEq × 10-3)		
	Isotonic Lavage	1/3 Isotonic Lavage	Isotonic Lavage
Lavage Fluid	1720	5420	0
Lymph	1 5	37 1 (2)	13 9
Mesenteric Venous Blood	5 4 (2)	9 8 (2)	1 9 (2)
Mesenteric Venous Plasma	13 6 (2)	25 7 (2)	4 1 (2)
Femoral Arterial Blood	0 8	1 6*	–
Femoral Arterial Plasma	2 3	4 1*	

*This sample withdrawn at end of the period of Na^{22} Lavage
Numerals in parentheses indicate the number of samples collected if greater than one, the specific activity given being the mean value

Unpublished data from experiments of E Grim J S Lee, and M B Visscher

TABLE XXVI

Data from Experiments on the Doubly Perfused Bullfrog Kidney Employing Isotopic Tracers for Water, Sodium and Potassium in the Renal Portal Vein Perfusate

Period	Renal Vein Outflow		Urine Flow ml/hr	Clearance		$[Na]_u$ mM/L	$[K]_u$ mM/L	D_2O moles % XS RPV = 1 00		SA Na^{22} K/min/mM RPV = 29780		SA K^{42} K/min/mM X 10^6 RPV 2 53		u/RV SA ratios		
	Actual Total ml/hr	Fraction RPV		Total ml/hr	% RPV			u	RV	u	RV	u	RV	D O	Na^{22}	K^{42}
1	718	77	12 50	13 80	00	49	0 65	784	759	10410	21660	1 19	1 84	1 03	481	647
2	492	68	11 48	12 81	00	55	0 60	714	639	11000	17410	0 90	1 20	1 12	632	750
3	527	71	11 83	14 00	00	62	0 90	620	685	8790	19950	0 79	1 59	905	441	497
			Perfusion with 002 M CN in RA started													
4	617	78	12 94	14 29	0 2	93	2 5	721	635	9345	19050	02	2 07	1 14	491	493
5	216	67	8 85	8 99	0 2	98	2 9	577	662	12000	19330	0 50	1 76	87	621	451
6	103	48	6 79	6 78	0 4	104	2 6	—	428	3509	13950	0 79	1 29	—	252	612

Unpublished data by T Hoshiko R E Swanson and M B Visscher

particularly by the use of a double glomerular filtration rate estimation Using inulin for one vascular source, and creatinine for the other, one can determine the relative contributions of renal artery and renal portal vein perfusate to the total glomerular filtration, and can correct, thereby, any values for appearance of various materials in the urine

If one puts creatinine and inulin in the renal artery perfusate, the measured glomerular filtration rates are very nearly the same, although there is consistently in our hands about a five per cent discrepancy with the creatinine higher than the inulin glomerular filtration rate If one places both of them in the renal portal vein perfusate, the discrepancy becomes proportionately very great There can be anywhere from zero to a very small glomerular filtration rate measured with inulin from the renal portal system, but there is always a significant excretion of creatinine We interpret this to mean that there is some tubular transport of creatinine in the frog kidney Whether this is secretion or leakage, I cannot say Perhaps others may have some ideas on that score In general however, the experiments which I am going to talk to you about are those in which less than 1 per cent of the glomerular filtration rate was attributable to the renal portal vein, (sometimes as little as 0 1 per cent) as measured by inulin clearances from the renal portal venous blood

Bearing in mind, then, the fact that the bullfrog kidney was perfused with a saline Ringer's fluid, which may induce certain significant changes, let us look at the data to see what they may, or may not, mean in connection with problems of renal secretion Table XXVI presents the results of a representative experiment The rates of perfusion, and urine flow in this particular experiment, indicate that about two per cent of the perfusate appeared as urine The glomerular filtration rate indicated as "clearance," compared with urine flow, shows the relatively small reabsorption of water I call attention to this because some of you are going to be very critical of these small differences between the total clearance and the urine flow This is a point which I raise particularly in relation to the question as to whether this can be considered to be a normal kidney However, the concentrations of sodium in the urine were fairly low — 49, 55, 62 mEq of sodium per liter — as compared with the 110 or so in the perfusion fluids in other words, half of the sodium was being reabsorbed I mention this because it indicates that the kidney was not dead, by any means It was also reabsorbing potassium, because the potassium

TABLE XXVI

Data from Experiments on the Doubly-Perfused Bullfrog Kidney Employing Isotopic Tracers for Water, Sodium and Potassium in the Renal Portal Vein Perfusate

Period	Renal Vein Outflow		Urine Flow ml/hr	Clearance		$[Na]_u$ mM/L	$[K]_u$ mM/L	D_2O moles % XS RPV = 1 00		SA Na^{22} λ/min/mM RPV = 29780		SA K^{42} λ/min/mM X 10 6 RPV 2 53		u/RV SA ratios		
	Actual Total ml/hr	Fraction RPV		Total ml/hr	% RPV			u	RV	u	RV	u	RV	D O	Na^{22}	K^{42}
1	718	77	12 50	13 80	00	49	0 65	784	759	10110	21660	1 19	1 84	1 03	181	647
2	492	68	11 48	12 81	00	55	0 60	714	639	11000	17410	0 90	1 20	1 12	632	750
3	527	71	11 83	14 00	00	62	0 90	620	685	8790	19950	0 79	1 59	905	441	497
			Perfusion with 002 M CN in RA started													
4	617	78	12.91	14 29	0 2	93	2 5	721	635	9345	19050	02	2 07	1 14	491	493
5	216	67	8 85	8 99	0 2	98	2 9	577	662	12000	19350	0 80	1 76	87	621	454
6	103	48	6 79	6 78	0 4	104	2 6	—	428	3509	13950	0 79	1 29	—	252	612

Unpublished data by T Hoshiko R E Swanson and M B Visscher

concentration in the perfusate was about 2 mEq per liter, and the urine potassium concentration was between 6 and 8 mEq per liter

In the perfusate that passed through the renal portal vein and did not enter the glomeruli to any significant extent, there was 1 mole per cent excess of D_2O In the urine as collected, there were 78 724 and 602 moles per cent excess of D_2O in the three periods Now this D_2O did not get to the urine by glomerular filtration, that is if we assume that glomerular filtration can be measured with inulin It must have come from the peritubular capillaries We do not know what the concentration of D_2O in the peritubular capillaries is, because unless Dr Oliver can tell us some way in which we can determine exactly how fluid distributes itself between the renal artery and renal portal vein systems by the time it reaches the peritubular capillaries we do not know how anyone could get a value for peritubular D_2O concentration

However what we have done is this we collected the renal vein outflow, which, as you can see, is bound to be a mixture of the effluents from the renal artery and the renal portal vein systems and as a first approximation we made the tentative assumption that the peritubular D_2O concentration was not too far from the renal vein concentration If we do make such an assumption, we find that the equilibrium approached is not too bad

I have shown results of one experiment because I thought it would be easier to think about a single experiment than about averages But I want to warn you that we do have instances in which the urine moles per cent excess for D_2O is higher than the renal vein moles per cent excess I have a suspicion that the assumption which I have been talking about is not always valid, strictly speaking It is not much higher, but it is high enough so that it is outside the range of experimental error for the measurements

In the case of sodium we put into the renal portal vein perfusate some Sodium 22 (29 000 counts per minute per milliequivalent of sodium) In the urine as formed there were 10 000, 11,000 and 9,000 counts per minute per milliequivalent of sodium and in these three periods the renal vein counts were more than twice as high

With regard to potassium approximately the same thing occurred except that the urine approached the renal vein potassium a little more closely than the sodium did Thus it can be said that the percentage of approach toward equilibrium in this experiment with respect to potassium was higher than for sodium The urine renal

vein ratio for D O was practically 1 for sodium about 5 and for potassium about 6

If in the subsequent periods 002 molar sodium cyanide is placed in the renal artery fluid we find that the sodium reabsorption was greatly diminished but not abolished Potassium reabsorption was greatly interfered with and we did not have any very significant change in the moles per cent excess of deuterium We hesitate to explain positively the effects on sodium and potassium but one can say that the movement was not abolished by cyanide movement from the peritubular space into the tubular urine continued and although the specific activities were different there was too much variability to permit us to say whether there was any change I wish I did not have to make such a negative statement because it is a rather crucial question

...ssing Is this type of poisoning reversible?

Visscher We have not tried it

Mudge Have you tried mercury?

Visscher Not as yet We have a lot of poisons on our list but as you can readily understand this is not an easy experiment to carry out

Oliver Did I understand you to say that you put the cyanide only into the glomeruli by way of the artery?

Visscher That's right

Oliver Why didn't you put it in the renal portal blood in the tubule?

Visscher Well for a purely technical reason It was simply because we did not want to change the concentration of our isotopic tracers which were only in the renal portal vein Now it is going to be a much harder job to set up the experiment the other way around because we have to juggle our concentrations But it can be done and should be done

Oliver Would a little sugar in the perfusate interfere with anything?

Visscher No I don't think so

Oliver It gives a good test of tubular integrity We always used to put sugar in the perfusion fluid just to check on whether the tubules were working (1)

Visscher I see We hadn't thought of using a second criterion

In Table XXVII are shown the results of a sample experiment in which D O was placed in the renal portal vein perfusate It will be noted that the D_2O in urine was 120 to 160 times the amount which could have been filtered with inulin through glo

TABLE XXVII

A Representative Experiment Showing the Excess of D_2O in the Urine over the Amount Filtered (u/gf) and the Approach to Equilibrium with the Renal Vein Effluent (u/rv) When D_2O is Placed Only in the Renal Portal Vein Perfusate

Period	RV Outflow		Urine Flow ml/hr	Clearance		Urine Sodium mEq /L RA 117 RPV 119	D_2O (Moles % Excess) RPV 1 00		D O Moles % Excess Ratio	
	Total ml/hr	% RPV		Total ml/hr	% RPV		u	rv	u/gf	u/rv
1	246	45	14 4	16 8	0 3	85	0 35	0 43	120	0 81
2	256	45	15 7	17 7	0 2	89	0 33	0 43	160	0 77
3	216	47	12 6	13 6	0 2	97	0 30	0 43	130	0 70
4	204	48	12 3	13 3	0 3	102	0 32	0 44	130	0 73

Unpublished data from experiments of Swanson, R E, Hoshiko, T, and Visscher, M B

meruli In this case the total glomerular filtration (clearance) was about six per cent of the perfusion rate

In Table XXVIII are some additional data on the effect of cyanide poisoning, which show the same appearance of isotopes in the urine in the poisoned kidney This occurs quite regularly even though sodium reabsorption by the tubule has been greatly affected, as can be seen from the Table

Pitts Dr Visscher, it was my impression, although you didn't say so, that there was no real change in the figures for water, sodium or potassium

Visscher I certainly wouldn't want to say definitely

Pitts What happened in the average for all experiments?

Visscher There was no change However, I should like to call attention to the fact that there was considerable variability, and we do not consider the absence of change to be proved

Oliver Why didn't you put the cyanide in the renal vein so that you would obtain a maximum tubular perfusion of your poison? When you put it in the artery, you may have been hitting just the tips of the tubules, a part which isn't too important

Visscher It is altogether possible Now, perhaps we could use some other criteria, such as absorption of sugar or urea

Oliver I think sugar would be much easier to use, and there would be a greater difference in functional activity between normal and damaged tubules

Pitts You could do it at the same time

Oliver Yes but I don t think the difference in functional effect would be as large, would it, if the urea were used?

Forster To my knowledge simultaneous concentration gradients for urea and glucose have not been determined in the frog

Thorn You indicated that the differences in sodium change were not significant In the next table, however, sodium decreased and potassium increased Would you consider that a significant difference?

Visscher Yes I would

Thorn Was that true in all experiments?

Visscher No, it was not, that is the trouble

Dock There is no objection to putting the cyanide in both perfusates, is there?

Visscher No

Dock You really don't know where you are until you do that

Visscher No, but let me explain one thing The reason we thought that cyanide placed in the renal artery would be adequate

was because if it is put there, it gets into the glomerular filtrate and should poison the tubule along its length Now, we know that the tubule was poisoned, because sodium reabsorption was either abolished or greatly diminished In other words, it was evident that there was some poisoning, there is no question about that Whether it was totally poisoned or not, we do not know

Oliver Aren't these assumptions contradictory? In your normal experiments you assumed that substances perfused through the glomeruli would not disturb the tubules sufficiently to interfere with the experiment and then, when the poison was given by perfusion of the glomeruli, you assumed it would affect the tubules

Visscher No, as a matter of fact, we have done the experiment in reverse with regard to D_2O and sodium, and have put the D_2O and sodium in the renal artery I did not show any of those data because they really do not add anything except to answer this question *If one puts the D_2O, or sodium in the renal artery and* there is no tracer in the renal portal system, one finds just as in

nt excess of D_2O and the
that of the renal vein by
not remain at the values

they had in the renal artery in other words, whichever way you do it you get the same result It means that water sodium and potassium, the three things we have measured which get into the glomerulus through the renal artery or the particular molecules or ions that get through are not exclusively those that eventually appear in the urine, but have been equilibrated one way or another with molecules and ions of the same species

Taggart Even assuming that the cyanide is as diffusible as D_2O, I wonder if the cyanide level isn t a little borderline Most of the transport systems need nearly *M/1000 for complete inhibition* Your final concentration here must have been close to M/2000

Visscher Yes The gut is pretty badly hit by M/1000 Perhaps we haven't yet done exactly what we ought to do, I am quite willing to concede that

Forster Was this done on *Rana catesbeiana?*

Visscher Yes, the bullfrog

Forster I think there is cause to examine your control preparation on the basis of some observations which Bodil Schmidt Nielsen and I made this past summer on *Rana catesbeiana* (2) We have been worried about the possibility that kidney function values observed in frogs, maintained for long periods in the laboratory or exposed to trauma accompanying perfusion experiments, may

meruli In this case the total glomerular filtration (clearance) was about six per cent of the perfusion rate

In Table XXVIII are some additional data on the effect of cyanide poisoning, which show the same appearance of isotopes in the urine in the poisoned kidney This occurs quite regularly even though sodium reabsorption by the tubule has been greatly affected, as can be seen from the Table

Pitts Dr Visscher, it was my impression, although you didn't say so, that there was no real change in the figures for water, sodium or potassium

Visscher I certainly wouldn't want to say definitely

Pitts What happened in the average for all experiments?

Visscher There was no change However, I should like to call attention to the fact that there was considerable variability, and we do not consider the absence of change to be proved

Oliver Why didn't you put the cyanide in the renal vein so that you would obtain a maximum tubular perfusion of your poison? When you put it in the artery, you may have been hitting just the tips of the tubules, a part which isn't too important

Visscher It is altogether possible Now, perhaps we could use some other criteria, such as absorption of sugar or urea

Oliver I think sugar would be much easier to use, and there would be a greater difference in functional activity between normal and damaged tubules

Pitts You could do it at the same time

Oliver Yes but I don't think the difference in functional effect would be as large, would it, if the urea were used?

Forster To my knowledge simultaneous concentration gradients for urea and glucose have not been determined in the frog

Thorn You indicated that the differences in sodium change were not significant In the next table, however, sodium decreased and potassium increased Would you consider that a significant difference?

Visscher Yes, I would

Thorn Was that true in all experiments?

Visscher No it was not, that is the trouble

Dock There is no objection to putting the cyanide in both perfusates, is there?

Visscher No

Dock You really don't know where you are until you do that

Visscher No, but let me explain one thing The reason we thought that cyanide placed in the renal artery would be adequate

was because if it is put there it gets into the glomerular filtrate *and should poison the tubule along its length Now we know that* the tubule was poisoned because sodium reabsorption was either abolished or greatly diminished In other words it was evident that there was some poisoning there is no question about that Whether it was totally poisoned or not we do not know

Oliver Aren t these assumptions contradictory? In your normal experiments you assumed that substances perfused through the glomeruli would not disturb the tubules sufficiently to interfere with the experiment and then when the poison was given by per fusion of the glomeruli you assumed it would affect the tubules

Visscher No as a matter of fact we have done the experiment in reverse with regard to D_2O and sodium and have put the D_2O and sodium in the renal artery I did not show any of those data because they really do not add anything except to answer this question If one puts the D_2O or sodium in the renal artery and there is no tracer in the renal portal system one finds just as in the opposite case that the moles per cent excess of D_2O and the specific activity of the sodium approach that of the renal vein by the same fractions — 9 and 5 — and do not remain at the values they had in the renal artery in other words whichever way you do it you get the same result It means that water sodium and potassium the three things we have measured which get into the glomerulus through the renal artery or the particular molecules or ions that get through are not exclusively those that eventually appear in the urine but have been equilibrated one way or another with molecules and ions of the same species

Taggart Even assuming that the cyanide is as diffusible as D_2O I wonder if the cyanide level isn t a little borderline Most of the transport systems need nearly M/1000 for complete inhibition Your final concentration here must have been close to M 2000

Visscher Yes The gut is pretty badly hit by M/1000 Perhaps we haven t yet done exactly what we ought to do I am quite willing to concede that

Forster Was this done on *Rana catesbeiana*?

Visscher Yes the bullfrog

Forster I think there is cause to examine your control prepara tion on the basis of some observations which Bodil Schmidt Nielsen and I made this past summer on *Rana catesbeiana* (2) We have been worried about the possibility that kidney function values observed in frogs maintained for long periods in the laboratory or exposed to trauma accompanying perfusion experiments may

meruli In this case the total glomerular filtration (clearance) was about six per cent of the perfusion rate

In Table XXVIII are some additional data on the effect of cyanide poisoning, which show the same appearance of isotopes in the urine in the poisoned kidney This occurs quite regularly even though sodium reabsorption by the tubule has been greatly affected, as can be seen from the Table

Pitts Dr Visscher, it was my impression, although you didn't say so, that there was no real change in the figures for water, sodium or potassium

Visscher I certainly wouldn't want to say definitely

Pitts What happened in the average for all experiments?

Visscher There was no change However, I should like to call attention to the fact that there was considerable variability, and we do not consider the absence of change to be proved

Oliver Why didn't you put the cyanide in the renal vein so that you would obtain a maximum tubular perfusion of your poison? When you put it in the artery, you may have been hitting just the tips of the tubules, a part which isn't too important

Visscher It is altogether possible Now, perhaps we could use some other criteria, such as absorption of sugar or urea

Oliver I think sugar would be much easier to use, and there would be a greater difference in functional activity between normal and damaged tubules

Pitts You could do it at the same time

Oliver Yes, but I don't think the difference in functional effect would be as large, would it, if the urea were used?

Forster To my knowledge simultaneous concentration gradients for urea and glucose have not been determined in the frog

Thorn You indicated that the differences in sodium change were not significant In the next table, however, sodium decreased and potassium increased Would you consider that a significant difference?

Visscher Yes, I would

Thorn Was that true in all experiments?

Visscher No, it was not, that is the trouble

Dock There is no objection to putting the cyanide in both perfusates, is there?

Visscher No

Dock You really don't know where you are until you do that

Visscher No, but let me explain one thing The reason we thought that cyanide placed in the renal artery would be adequate

was because if it is put there it gets into the glomerular filtrate and should poison the tubule along its length Now we know that the tubule was poisoned because sodium reabsorption was either abolished or greatly diminished In other words it was evident that there was some poisoning there is no question about that Whether it was totally poisoned or not we do not know

Oliver Aren t these assumptions contradictory? In your normal experiments you assumed that substances perfused through the lomeruli would not disturb the tubules sufficiently to interfere ith the experiment and then when the poison was given by per ion of the glomeruli you assumed it would affect the tubules

Visscher No as a matter of fact we have done the experiment verse with regard to D_2O and sodium and have put the D O and sodium in the renal artery I did not show any of those data because they really do not add anything except to answer this question If one puts the D_2O or sodium in the renal artery and there is no tracer in the renal portal system one finds just as in the opposite case that the moles per cent excess of D_2O and the specific activity of the sodium approach that of the renal vein by the same fractions — 9 and 5 — and do not remain at the values they had in the renal artery in other words whichever way you do it you get the same result It means that water sodium and otassium the three things we have measured which get into the lomerulus through the renal artery or the particular molecules ions that get through are not exclusively those that eventually appear in the urine but have been equilibrated one way or another with molecules and ions of the same species

Taggart Even assuming that the cyanide is as diffusible as D O I wonder if the cyanide level isn t a little borderline Most of the transport systems need nearly M/1000 for complete inhibition Your final concentration here must have been close to M/2000

Visscher Yes The gut is pretty badly hit by M/1000 Perhaps we haven t yet done exactly what we ought to do I am quite willing to concede that

Forster Was this done on *Rana catesbeiana*?

Visscher Yes the bullfrog

Forster I think there is cause to examine your control preparation on the basis of some observations which Bodil Schmidt Nielsen and I made this past summer on *Rana catesbeiana* (2) We have been worried about the possibility that kidney function values observed in frogs maintained for long periods in the laboratory or exposed to trauma accompanying perfusion experiments may

not be identical with those noted in animals kept under more or less normal conditions as far as ecological relationships are concerned. There are three differences between the intact frog in its natural environment and your control perfusion preparations so far as tubule function is concerned. In the first place, there is a considerably higher rate of tubular water reabsorption after the glomerular filtrate has been formed in the intact animal under optimal conditions. In contrast to the inulin U/P ratio of 1.1 noted in some of the perfused nephrons, frogs in their natural environment may have ratios of 6, 8 or perhaps even as high as 10, that is, even when the animal is totally immersed and maximally hydrated.

Visscher You see, we are frankly admitting that what we call our buffered ion solution may not be just what it ought to be in the frog nephron to do the job correctly. It is admitted that this is an abnormal situation.

Forster The second point I wanted to bring up was the matter of creatinine leakage through the tubule cells. I was struck, when we originally made these studies in 1938 (3), with the fact that the inulin clearance checked the creatinine clearance absolutely, and with wide variations in the hydration state of the frogs there was never any deviation from unity.

Visscher No deviation at all?

Forster Only random variations within the experimental error of five per cent or so. The mean creatinine/inulin clearance ratio is 0.996, standard deviation of 0.064.

Visscher There was as much distribution above as there was below 100 per cent?

Forster Yes.

Visscher That is where we come out differently. Considering twenty-four simultaneous clearances through the renal artery of inulin and creatinine in only two was the ratio below 1. In all the others it was above 1, the highest value above 1 was 1.08, and the lowest value .87.

Forster The identity of the inulin and creatinine clearances has recently been verified in *Rana catesbeiana* and *Bufo marinus* by the Sawyers (4) at Harvard so I think that is another indication of a difference between your perfusion setup and renal function in the intact animal. The *Bufo marinus* creatinine/inulin clearance ratio is 1.04, ± 0.066.

The third difference is that while intact frogs do lose a little sodium and chloride in the urine, normally it is practically distilled water, with essentially all the filtered sodium and chloride

being reabsorbed by the tubule cells Average salt concentration in urine of summer frogs is 4 6 mM So it seems to me that there are three aspects of your control preparation which indicate that the nephron has been disturbed somewhat physiologically namely, the fact that the tubules are reabsorbing essentially no water, that they are leaking creatinine, and that they are failing to reabsorb at least 50 per cent of the sodium and chloride which one would expect

Visscher You probably know that Hogben and Bollman in Rochester did some creatinine inulin clearances with the bullfrog, and found on the average that this ratio was 1 27 in their preparations This may fall in line with what you are saying that they had even more abnormal preparations than we had

Pitts Have you perfused the renal portal vein with creatinine to indicate secretion?

Visscher Both inulin and creatinine were put in the renal portal vein perfusate

Pitts And you got no inulin in the urine, but some creatinine?

Visscher That's right

Pitts What happens in that preparation if you put the same amount of cyanide in?

Visscher We haven't done it

Bott When these experiments were reported last spring at the Federation meetings (5), I made the criticism that the concentration ratios did not seem to be high enough and certainly in some cases I should still say so However, I looked up some of our double perfusion experiments and found that according to our results, it was very difficult to get the concentration ratio of inulin very high under these conditions I suggested, in talking with Mr Hoshiko, that the renal perfusion pressures were a little higher than seemed necessary, especially the renal portal perfusion pressure I wondered if reducing these might not make the ratios more normal I suggested, also, that some other determinations, such as chloride, be made Do you know whether he tried these suggestions?

Visscher Not as yet, but it is on our schedule to do both of those things, Dr Bott They were very good suggestions

Bott I think Dr Oliver's suggestion about the sugar was excellent, but what about the sodium concentration of the perfusate? Have you found an average figure of 110? I think that is what you said

Visscher I know it is low

Bott Yes It is lower than in the mammal, but it seemed to me that my average figures were a little higher than that *

Visscher Several values in that range — 100, 110 or 120 — have been used, and no significant differences were seen

It might be of some interest to report on observations made on the quantitative relationship between glomerular filtration and volume of urine flow in frog kidneys doubly perfused, in which a solution hypertonic to that used in the renal artery was put into the renal portal vein A typical experiment is seen in Table XXIV It will be noted that when 10 per cent mannitol was added to the perfusate for the renal artery, the urine flow figures became higher than the measured glomerular filtration (clearance) values Thus a net transport of water into the tubules was indicated

Of course this finding may not be surprising but, nevertheless we thought it interesting The same directional changes occur if one uses five per cent mannitol We might have expected that the urine would contain water that was drawn in by osmotic forces nevertheless, we thought it worth while to make some measurements to confirm it

Pitts Dr Visscher, do you use any colloids at all in either of your perfusing solutions?

Visscher No at least, we haven't so far

Pitts Do you have any reason for not doing so? Do you think you could possibly establish more nearly normal urine flow and filtration rate relationships, by adding colloids of some variety to both your circulations, especially to the renal portal venous circulation?

Visscher It is possible that a more nearly normal preparation could be obtained

Selkurt Dr Forster, in terms of your own experience, and as a comparative figure to these data what are the normal filtration rates in these bullfrogs?

Forster Well it is very difficult to characterize 'normal" circulation in a cold blooded animal, and especially in a semiaquatic form such as the frog This species (*Rana catesbeiana*) is primarily aquatic, but spends a good deal of time sitting out of water on lily pads, as I know from direct experience The water balance problem differs radically in those two circumstances A third circulation norm is introduced into many renal clearance studies when heavy hydration is induced by the injection of large quanti

*A search of my notes disclosed an average of 102 mEq per L. for bullfrog serum sodium

TABLE XXIX

The Effect of Perfusing a Solution made Hypertonic with Mannitol through the Renal Artery upon the Net Movement of Water across the Renal Tubule in the Doubly Perfused Bullfrog Kidney

	RV Outflow		Urine Flow	Clearance		Urine Sodium
	Total ml/hr	% RPV	ml/hr	Total ml/hr	% RPV	mEq /L (RA 121)
#57 (a)	105	55	4 90	5 79	0 1	73
(b)	48	57	4 90	5 86	0 3	75
	Switch to 10% Mannitol in Ra					
(c)	92	68	4 29	4 30	0 2	65
(d)	90	86	3 68	1 68	12	43
(e)	75	87	2 50	1 04	18	44
(f)	48	87	0 56	0 25	26	50
						(Ra 119)
#58 (a)	341	32	5 73	8 39	0 2	46
(b)	392	25	8 39	12 1	0 2	60
(c)	377	26	9 72	12 9	0 2	71
	Switch to 5% Mannitol in Ra					
(d)	116	53	2 95	3 06	1	70
(e)	131	24	5 26	4 13	2	81
(f)	142	36	7 04	5 15	2	83
(g)	124	25	5 44	4 00	2	86

From unpublished experiments of Swanson R E Hoshiko T and Visscher M B

ties of fluid beyond the skin barrier that is, directly into the dorsal lymph sac The urine flows in Dr Visschers perfused preparation are similar to those that have been obtained in the past when large quantities of fluid are injected under the frogs skin Filtration rates are normally higher than this in the intact animal but the glomerular filtration rate and urine flow closely approximate one another I commented on that aspect of heavy hyd

Pitts Dr Visscher you have awfully low filtration fractions do you not if you consider the arterial perfusion? Even there you get rather low filtration rates considering the volume of fluid perfusing the renal artery alone

Dock Ten per cent in the ones mentioned earlier where the data were given

Pitts I thought they were somewhat less than that

Visscher Well five to ten per cent in the best cases

Forster About five to ten per cent of the renal plasma flow is filtered in the intact frog that is of blood arriving via both the renal portal vein and the renal artery In heavily hydrated intact bullfrogs renal plasma flow was 700 ml per kg per min when the simultaneous glomerular filtration rate was 42 ml per min and hence the filtration fraction was 0 06 (6)

Pitts Couldn t you solve your problem by bringing the frog back under the conditions which result from sitting on a lily pad if you just lowered the pressures and ended up with less glomerular filtration?

Visscher That is what Dr Oliver suggested this morning

Oliver We found that with what was apparently a good preparation we could make sugar disappear or reappear in the urine simply by lifting the arterial bottle or lowering it and so speeding or slowing the rate at which filtrate was formed

Pitts I thought these filtration rates were per kilogram per hour

Visscher No these are absolute numbers for the animal

Pitts Well they are roughly four times the normal

Selkurt What was the portal venous perfusion pressure? I don t think you cited it

Visscher It was adjusted in different experiments so as to obtain approximately 50 50 flows through the two circuits As I recall it was something like 50 centimeters of water for the artery and 15 or 20 for the portal I do not have the tabulation here

Forster We might consider the situation which exists in the aglomerular kidney of certain marine fish where a double perfusion setup is not required to study discrete tubular functions The entire kidney in such a form as the goosefish is made up of nephrons one very much like another composed solely of epithelium similar to that of the proximal convoluted segment of other vertebrate kidneys and undifferentiated along its entire length down to the area of the collecting ducts The renal tubule is supplied solely by a venous circulation so there is a simple arrangement in which the peritubular circulation under low pressure is

presenting the normal plasma constituents to a single layer of cells

Urine obtained from freshly caught goosefish is essentially sodium- and chloride-free The magnesium and sulfate content, however, may be a hundred times greater than that of the blood Such urine, despite the deficiency of univalent ions, approaches isotonicity with the plasma but does not quite achieve it The urine is always hypotonic to blood in both glomerular and aglomerular marine fish, and from a teleological standpoint this is a perfectly ridiculous situation because here are animals living in a hypertonic medium whose kidneys are saving salt, while the fish are drinking sea water But I think this serves to demonstrate the complexities of ion regulation in a system, which is morphologically as simple as anything we have in renal physiology Presumably highly diffusible univalent ions are on one side of the single cell barrier and don't appear on the other side at all, and on the other hand the divalent ions are concentrated against a tremendous gradient Furthermore the urine is hypotonic to the plasma and a pressure is built up in the ureter which is higher than that of the venous pressure

Darrow Hypotonic? How do you estimate that?

Forster By freezing-point depression There is about an atmosphere, or 0 1 of a degree C difference between venous blood and urine * Average freezing point of *Lophius piscatorius* urine is −0 562° C, and plasma is −0 665 I don t know how this should be interpreted It is rather difficult to envisage a diffusion process at one level of the tubule which would carry across sodium and chloride, and then have reabsorption of these ions at another level, because there is nothing like proximal and distal segments, or any other evidence of morphological differentiation, along the length of these aglomerular tubules On the other hand, it is equally awkward to conceive of a barrier with respect to sodium and chloride transport when the divalent ions can get through as fast as they do

Another strange thing that happens to aglomerular and other marine fish, is that once they are captured and maintained in the laboratory, sodium and chloride floods into the urine, they exhibit a progressive diuresis, and the urine becomes isotonic with blood and remains so, as the total electrolyte content of blood continues to rise

*Forster, R P A comparative study of renal function in marine teleosts (Unpublished data

The only explanation I can give as to why renal tubules of marine fish save salt, is in terms of teleost evolution Pale ontological and other evidence, advanced by Marshall and Smith (7), indicates that they spent their early evolutionary history in fresh water, and although these forms have now gone into the ocean, and have made suitable adjustment with respect to water conservation via reduction of glomerular activity, the tubules haven t heard about it yet and are going on saving sodium and chloride as they did in the old freshwater days

Darrow But are your fish in the ocean?

Forster Yes, when they are observed over some period of time they are maintained either in livecars out in the bay, or in large tubs supplied with running sea water in the laboratory

Berliner Don't you think you ought to mention in that regard that the sodium and chloride probably come out in large amounts through the gills?

Forster Yes Smith showed long ago (8), and subsequent studies have confirmed the fact, that sodium and chloride, along with ammonia, are actively eliminated by the gills of marine fish Marine fish drink hypertonic sea water continually and absorb considerable quantities of it, but high sodium and chloride concentrations do not build up in the blood because the gills normally get rid of these univalent ions as fast as they are absorbed from the gut Plasma chloride concentration is relatively stable at 175 mEq per L in Lophius The kidney's role in electrolyte excretion consists mainly of eliminating the divalent ions

Oliver Why should they change when you simply keep them in a pen?

Forster Grafflin has called attention to osmotic difficulties following skin damage, incurred in ' maintenance, as the initiating event (9)

Pitts I don't think you r? You say a salt-water fish is d true, but it doesn't ' tory, it begins it, pick it up, is a different fi

Forster I d' Pitts says is p caught, they associated with composition of

ance of significant quantities of sodium and chloride in the urine

Bott Do they go on living as normal animals?

Forster Physiologically, no marine fish is a normal animal once it has been caught, but they are active and look fine These aglomerular goosefish weigh fifty pounds or so and it is a tremendous job restraining them for urine and blood collections even after they have been maintained in tubs for 10 days or so They seem to be in good condition in every way, except for the criteria which we associate with physiological irregularity, mainly diuresis, and the presence of sodium and chloride in urine The gut is filled with fluid, and the divalent ion concentration in the plasma rises even though the total amount excreted is increasing This could only mean increased drinking and absorption of sea water What causes the increased drinking is another matter

Pitts Presumably, the gills can't handle the large amount of salt which is acquired as a consequence of increased rate of drinking

Visscher Dr Forster, what is the composition of the fluid at the head end of this tubule?

Forster I wish I knew

Visscher The reason I ask the question is this that there are very few membranes I know of which are permeable to divalent ions, and impermeable to monovalent ions It is true that one could imagine a kind of carrier system which would move divalent and not monovalent ions Is that right, Dr Ussing?

Ussing It sounds likely

Visscher On the other hand there is nothing very difficult about quite another assumption namely, that at the head end of the aglomerular nephron, the fluid in the peritubular space passes in by some mechanism, let's say electro osmosis, which moves this stream of fluid with the sodium chloride, magnesium and sulfate Then, if farther along the tubule there is a different permeability, more characteristic of many membranes such as the gut, in which or across which, divalent ions of both signs move with great difficulty but monovalent ones move with much greater ease the sodium chloride might move out with the stream of fluid and there would be a fluid circuit that would accomplish everything including the production of hypotonicity

Pitts Haven't you merely described aglomerular glomerular activity?

Bott Inulin is not excreted by this fish is it? I know the toadfish doesn't excrete it so it could not be that exactly

Pitts No, the aglomerular fish excretes no ferrocyanide, and no inulin

Oliver Is there no way of getting at the problem by puncture? Could you puncture the kidney and look at it afterwards to see where the sample had come from?

Forster It could be done, but we haven't tried it The main reason I introduced these facts concerning the aglomerular tubule was to emphasize that we should recognize the differences between animal membranes with respect to the distribution of ions Even in carrying our observations from one vertebrate nephron to another, we can make some serious mistakes if we assume that a tubule "is a tubule is a tubule" While there are some very persistent general functions which are shared by all vertebrate nephrons, such as glucose reabsorption and phenol red or *p*-aminohippurate secretion, there are also striking variations in tubular function noted in comparing even closely related species One should be very cautious in working with different animals to keep in mind the possibility of differences in function, even in what appears to be morphologically similar structures

Selkurt In other words, you don't have to assume tubular secretion of water in man, is that your point?

Forster Yes

Thorn It is interesting that in the uremic patient you may have an important rise in serum potassium as a late toxic manifestation As these fish die, do they have high or low serum potassium?

Forster As they die the total electrolyte content rises in the plasma, and the concentrations in the urine are parallel with the rise in the plasma We have not followed potassium concentrations in plasma and I know of none in the literature

Dock You never tied the gullet after catching the fish?

Forster As a matter of fact Grafflin did just that in a series of studies

Pitts They would still become dehydrated and die I think that is what Grafflin found They lose water osmotically through their skin Presumably, that is the reason why they drink

Dock I see It is the skin that is damaged first, from bruising perhaps? They thrash around a lot in a small space

Pitts Grafflin used to describe the UHH technique, that is, "unhandled by human hands," as being the only way to get a fish that resembles a normal fish in any way

Lotspeich How do you know they don't put out any sodium chloride normally?

Pitts Because when you catch a fish and collect the urine it has in the bladder, there is no sodium chloride in the urine But subsequent urine contains it Dr Ussing, would it be feasible to study potential differences in the punctured cells of these renal tubules using the microelectrodes in, say fresh caught fish? It is quite easy to isolate little bits of renal tubules in freshly caught fish and sick fish

Ussing It would be nice to try anyway Whether it would get is anywhere is hard to say But what happens if one brings a little odium chloride into the tubule? It really would answer Dr isscher's question, would it not? In case sodium brought into the bule is reabsorbed, then his proposal could be upheld

Forster Yes I mentioned Dr Visscher's suggestion as one of possibilities namely, that we have a diffusion of all the ions ne level of the tubule, and a selective reabsorption of the mono nt ions at another Another possibility is that there is a barrier e transport of monovalent ions I imagine that there are ways tting at these alternatives through micropuncture techniques but I fear that by the time one got down into the kidney and had needles sticking through the tubule it would be acting pretty much like the kidney of the relatively undisturbed fish on the second day following capture

Ussing That is true And that is also what would bother one even if one could measure the potentials They probably would not be like the intact ones

Pitts Dr Forster, in connection with that, have you ever noticed any difference in the capacity of tubules to concentrate PAH or phenol red, taken from freshly caught fish and from those that have been kept in the livecar for a week?

Forster No, this electrolyte leakage is entirely independent of the secretory process involving *p* aminohippurate and phenol red The kinetics of the latter system are undisturbed as time goes on and as the fish becomes progressively diuretic

Thorn Have you ever injected desoxycorticosterone into these fish?

Forster No, I have not

Steinbach What is the relative rate of urine formation in freshly caught fish and fish that have been injured?

Forster The urine flow goes up about twenty fivefold in the production of the diuresis The goosefish forms tremendous quantities of fluid through tubular action At the height of diuresis, urine flows of 75 ml per Kg per day were noted in goosefish,

the flow in normal undisturbed fish with chloride free urine is probably less than 3 ml per kg per day This tendency to form large quantities of urine by tubule activity when there is a high urinary electrolyte content is reminiscent of Visscher s observation in the perfused frog where the addition of osmotically active substances induced urine flows higher than simultaneous glomerular filtration rates Completely glomerular marine fish such as the sculpin also exhibit urine flows exceeding glomerular filtration rates when they are maintained several days in diluted sea water presumably because additional fluid is added to the filtrate by the tubules along with the transport of osmotically active univalent and divalent ions

Steinbach As the urine output rises does sodium chloride excretion increase?

Forster Sodium chloride concentration goes up with urine flow the salt concentration and the freezing point depression raise the urine volume

Dock Is it isotonic?

Forster Urine and plasma are isotonic during diuresis Urine is hypotonic only in the normal state In the diuretic state the urine suddenly becomes isotonic and remains isotonic throughout the progressive diuresis which we have followed for as long as ten days

Dock It remains acid?

Forster Yes you can t make marine teleost urine alkaline even by the injection of enough bicarbonate to kill it

Steinbach The whole behavior is consistent with a nonlethal quantitative change isn t it? It certainly doesn t bother the fish All the fish marking experiments which are done include handling the fish and you catch them again three or four years later still alive and active If there is greatly increased urine flow and the appearance of salt it appears to me the obvious hypothesis is similar to one that Dr Visscher postulated

Taggart Doesn t that imply that all the things which are excreted solely by glomerular filtration in other kidneys are here completely reabsorbed?

Visscher No I object strenuously to that interpretation There is no reason whatever to suppose that the permeability of the region into which let us say the sodium chloride enters is identical with the permeability of the glomerulus

Dock Dr Wallace do you think that when sweat is being ex

creted normally, sodium is being reabsorbed as it comes down the duct?

Wallace That would be my interpretation of the fact that, as the rate of sweating increases, the higher the sweat sodium concentration becomes with a given level of adrenal activity Is that right, Dr Thorn?

Thorn Yes, I think so

Visscher Isn't it the greater the rate, the higher the level?

Wallace Yes, the greater the rate, the higher the level of chloride and sodium, and the lower the concentration of potassium in sweat

Pitts Aren't you rather begging the question? What difference does it make which way fluid is going through the cell? In one direction it separates out ions and water and in the other it does the reverse, so why do you necessarily need to emphasize that?

Dock That is what I was trying to get at I don't see that reabsorption is necessary in any of those places

Pitts The cells excrete faster, so they put out more nearly the concentration of plasma

Visscher Except, of course, that reabsorption does occur in cer
in places, as, for instance, in the gut

Dock We are not talking about the gut We are talking about
g necked glands like the salivary, where the secretion is done in
type of cell and then it runs along a duct You have to assume
it is being absorbed as it goes down that duct

Visscher We really do not know enough about the facts of the situation If somebody were to cannulate the individual duct, and find out what the composition of the secretion is at various levels in the duct, we should be in a much better position to talk about it

Dock Could this be done with radioisotopes and radio autographs to see what the concentration of sodium is in the cells and in the lumen?

Oliver The radioautographs are generally so diffuse that you
nnot localize things very well, at least judging from the prepara
ns I have seen of the kidney

itts With respect to magnesium phosphate, magnesium is put
in supersaturated solution Actually, with a good many fish,
ake them up out of the ocean and you can get crystalline acid
hate out of the urine I suppose, using radioactive phosphate,
could expect to get a radioautograph which might tell yo
g the length of that tubule whether, up towards th
is more dilute than it was down toward

Oliver It would depend on how long the tubule was There are such wide radiation effects

Dock It depends on how big the halo is, of course

Ussing Of course, phosphate has a big range It is really one of the worst

Dock I think that cannulating the salivary and other ducts would be a difficult job I do not believe any of those will be normal when you get to work on them

Pitts Fundamentally, that is what you want to know, isn't it? Is this urine more or less concentrated with *respect to any given* constituent at its head end or its tail end?

Dock How long are these nephrons?

Oliver How big is the fish?

Forster In a fish that weighs about fifty pounds, the kidney may be half as big as my fist

Dock But the length of the nephron must be several centimeters long You don t need to worry about halo effect there

Oliver If the tubule is a centimeter or so long perhaps you could stretch it out

Pitts Well, these things come all curled up

Oliver You can stretch them out

Forster There are no morphological markers either, as to what is proximal and distal It would be necessary to tease out carefully the entire length of a tubule in order to orient it

Dock Then they wouldn't be any good

Thorn If these animals die via the mechanism of ingesting sea water, has it been shown that they would live twice as long if sea water were diluted 50 per cent, or do they just drink more under these circumstances?

Forster I don't know

Steinbach Does calcium go along with magnesium? Is there a change in calcium concentration?

Pitts It is considerably lower, I think, than the magnesium

Steinbach That is, the ratio of urine to sea water concentrations *is not* the same for magnesium as for calcium?

Pitts It might be, but there is more magnesium than calcium

Steinbach I know that, but because of this mechanism which reabsorbs the monovalent ion, I was wondering whether you might expect all the ions to concentrate about the same percentage for a given value

Pitts It would just be concentrated sea water from which sodium and chloride had been removed, wouldn't it?

Pitts I have tried it on a mammalian kidney, and you cannot do it

Dock You mean you get too much edema formation, or what happens?

Pitts Yes, I think so I should like to ask Dr Berliner whether he ever gave 6063 to a dogfish, or to an aglomerular fish?

Berliner I should like to mention some experiments which Dr Henry Heineman, and Dr Jurg Hodler,* in Dr Homer Smith's laboratory, were doing in Maine this summer I haven't done them myself, I was only an interested bystander They gave 6063 to several species of fish In the dogfish the urine pH varied over a narrow range around a value of 5 8 and it was not shifted from this value by large amounts of either bicarbonate or 6063 However, there were some very interesting changes in the blood after 6063, which suggested interference with the transport of sodium ion by the gills, as well as some interference with the transport of CO_2, that is, the loss of CO_2 through the gills However, the *changes were not all due to failure to liberate CO_2 in the gills* They also gave 6063 to sculpins and found no effect on the urine, these were long horn sculpins which have appreciable filtration rates The situation was different in the catfish, a fresh water species which has a relatively much higher filtration rate and urine flow, more like the frog in that it always puts out a dilute urine containing practically no electrolyte When catfish were given 6063 the urine pH rose about one unit from around 6 to about 7 These fish were the only ones in the group Drs Heineman and Hodler studied, in which the urine became alkaline when bicarbonate was administered

Pitts I have given bicarbonate intravenously to dogfish until they became rigid and tetanic and still put out urine that was highly acid

Berliner Yes they gave large amounts of bicarbonate If the bicarbonate load without prac- ie plasma bicarbonate and pH l within an hour However, if they gave 6063 at the same time, the plasma bicarbonate did not come down within 24 hours

Dock Did they have carbonic anhydrase in their gills?

Berliner Yes, they did (10)

Bott I have been wondering whether any of the ion transport perts here would interpret for me those experiments of Wilbrandt 1) that were made in Philadelphia a number of years ago in

eineman H and Hodler J Personal communication

potential is measured? That *is what would be needed to evaluate the figures*

Pitts Dr Thorn has some data on intestinal transport of ions Would you care to comment?

Thorn Returning to the discussion of the hormonal effect on the intestinal tract I should like to report that Dr Kendall Emerson Jr, and Dr Dalton Jenkins, in our laboratory, have followed work along the same lines as Dr Benger and Dr Murray Steele, in New York in their study of the effect of adrenal hormone on sodium exchange in the intestinal tract Normally, of course, even in adrenal insufficiency, there is little sodium lost in the feces This is of great interest in view of the tremendous turnover of sodium which occurs normally in the intestinal tract We know that in patients with large quantities of sodium chloride in their body, such as the edematous nephrotic type of patient, the administration of an ion exchange resin by mouth does not produce any large quantity of sodium chloride in the stool It would appear, under these circumstances, that the resin is incapable of extracting sodium which is turned over in the intestinal tract On the other hand if sodium chloride intake by mouth is increased then the resin becomes quite effective in enhancing the sodium loss in the feces

I can now report a study of a patient with Addison's disease who was not being maintained on desoxycorticosterone acetate Under these circumstances, on a control diet the fecal excretion of sodium over a three day period amounted to approximately 20 milliequivalents for the entire period When a quantity of resin, approximately 45 grams per day was administered, the fecal sodium excretion rose tenfold to approximately 225 milliequivalents This would suggest that in the Addisonian patient, without adrenal compensatory mechanisms the resin was able to extract sodium chloride from the intestinal tract When the same experiment was repeated on desoxycorticosterone, there was a reduction in the total quantity of sodium chloride extracted from the intestinal tract by the resin The dose of desoxycorticosterone utilized was 10 mg This study has been reported by Dr Emerson (12) Considering the entire gastrointestinal tract, it is interesting to analyze the different sites of action of the various adrenal steroids We know that desoxycorticosterone can affect the sodium potassium ratio of the saliva and apparently under certain circumstances can affect the fecal loss of sodium and potassium We know also that the cortisone or hydrocortisone type of compound can increase pepsinogen secretion and give rise to an increased uropepsin excretion Dr Seymour

Gray and his group have demonstrated this effect with cortisone and with ACTH In addition more recent studies by Dr Joseph Dingman in our group Dr Paul Patterson formerly of the Childrens Medical Center, have demonstrated an increase in bile flow and in bile pigment elaboration following cortisone administration Thus it would appear that the adrenal steroids have a marked effect at various levels in the gastrointestinal tract and that the 17 hydroxy compounds are particularly effective in increasing gastric secretion bile secretion and perhaps pancreatic secretion and that the potent salt retaining factors are quite effective in altering the salivary sodium potassium ratio and the exchange of sodium and chloride in the lower bowel

Visscher In this connection I might recall the paper by Clarence Dennis and Earl Wood in 1940 in which they studied adrenalectomized dogs prepared surgically with Thiry Vella loops and measured quantitatively the capacity of those segments to absorb sodium chloride potassium and water They found just as you found in your Addisonian that sodium was actually lost to the intestinal segments in the hypoadrenal state when they were taken off cortin that potassium was still absorbed and that on treatment with cortin the situation was returned to normal I would suggest tentatively at least that in the normal state with relatively low sodium concentrations in the lower end of the bowel and relatively high potassium concentrations and with the types of ion exchange resins which are employed that you have relatively greater binding of potassium and sodium partly because of the low sodium content in the intestinal content

Mudge The sodium content of the bowel is very high isn't.

Visscher Yes but there is resin in the intestine in equilibrium with the fluid around it Exchange will be controlled mainly by what happens in the lower part of the bowel The exchange process is fairly rapid

Thorn Would you expect that the several grams of sodium chloride added to the patients diet would be reflected as added sodium chloride in the colon?

Visscher Yes I might if the effect were mediated through let us say adrenocortical secretion which alters the capacity of the lower end of the intestine to bring about this low sodium concentration

Dock McCance showed originally that if sodium is removed from the diet the sodium content of saliva gastric juice and other secretions falls promptly Presumably if sodium is added to the

diet, at some critical point the reabsorption of sodium from the lower part of the gut is reduced

Elkinton I might point out that the only cardiac studied in our laboratory with resin in whom we were able to remove a much larger amount of sodium than was in the diet, was a patient who had had a colostomy (13) He was not passing the resin by rectum, we were obtaining it from his upper colon He was the only one from whom we could take out approximately 150 to 160 mEq of sodium in twenty four hours, when the intake was only 20 or 30 But then if you say that there is a constant equilibration between the resin and the sodium content of the gut, I am still wondering what goes back into that resin when it comes out with such a small amount

Visscher Potassium, hydrogen ion and ammonium ion

Dock It was potassium in Steele's experiments

Berliner The increase in potassium did not equal the reduction in sodium excreted

Dock But it is mainly potassium that is added

Berliner To balance up some of the resin must come out in the hydrogen form.

Visscher There could be ammonium There has to be some cation

Berliner The resin is not actually necessary to produce the phenomenon It can be observed with the spontaneous electrolyte content of the stool *Drs J O Davis and D S Howell* in our laboratory have been working with dogs with experimental ascites The normal dog has a small amount of both sodium and potassium in its stools When ascites is produced, the sodium goes down and the potassium up When the adrenals are removed the sodium goes back up and the potassium down The absolute changes are small but the relative changes quite large

Steinbach To what degree can the intestine be used as a model of the kidney tubule in other words, what is the relationship?

Thorn I would be willing to speculate that the more we study the turnover of the electrolytes in the intestinal tract, and the factors which regulate this flux, the more analogies there will be between the secretory and reabsorptive aspects of the intestinal tract with those of the renal tubules

Pitts Fundamentally the reason for putting together a program of this sort was the belief that certain processes of transport, which can be identified in nerve and muscle cells, and observed in isolated renal tubular segments and in liver slices have elements in

common We felt that absorption in the gastrointestinal tract is related to absorption by the renal tubules In other words we felt that we as renal physiologists could learn from a multidisciplinary approach many things applicable to the kidney In certain instances simpler systems have been studied much more thoroughly and in a much more penetrating fashion than has the kidney I am certain that much of what has been said about cell function here can and will eventually be applied to renal function

Steinbach A second question is motivated partly by the fact that I teach elementary classes It is my recollection that if you run of earthworms and Crustacea and so on up to the nephrid look over the whole gamut of excretory devices from the nephrid the intestine the least common denominator of all those is the sodium movement Is that correct?

Forster Yes malpighian tubules of *fresh water* insects which resemble aglomerular renal tubules seem to form approximately isotonic urine Sodium reabsorption occurs subsequently in the rectum where the fluid becomes hypotonic before being eliminated (14) The nephridial tubules of the earthworm which are supplied with a nephridiostome, have been studied recently by Ramsay (15) They form a hypotonic urine in the distal segments and a tempting analogy has been drawn between the nephridium and the vertebrate nephron The situation in the marine invertebrates is quite chaotic at the present time but it seems that there is very little ionic regulation in any of them except perhaps the cephalopod Mollusca and the decapod Crustacea (16) A very recent paper by J A Ramsay on the excretion of sodium and potassium by the malpighian tubules of a blood sucking insect Rhodnius gives a detailed account of ionic regulation and water balance (17)

Steinbach It has interested me over a period of years that on a cellular basis the same phenomena can be duplicated in frog muscle fiber or nerve One of the main functions of cells in maintaining their internal environment and protoplasmic composition seems to be a sodium extrusion so that to get a secreting epithelium all one needs is an ordinary cell which is a little one sided

Pitts I have the impression that moths or some other variety of insect have the peculiarity of producing potassium in large amounts in their extracellular fluids and practically no sodium Is that right?

Ussing That is true for many insects

Pitts What happens to their cells? What do they have to have in their cells — sodium?

Steinbach There is a division between herbivorous and carnivorous insects on that The carnivorous insects function the way we do, and the herbivores have very high potassium A few measurements have been done on tissues, and the tissues (muscle) are quite like mammals with respect to sodium and potassium

Dock In the cells?

Ussing Have you seen the paper by Hoyle (18), in which he states that if you inject beneath the nerve sheath of an insect the same high potassium concentration found in its blood, it will block the nerve? Evidently there is a very thin layer along the nerve underneath the nerve sheath, which has a composition quite different from that of the insect blood, and rather like our own tissue fluid with its low potassium concentration When high concentration potassium solution is injected into this space, the nerve is blocked Perhaps the difference between vertebrates and insects is not so great as it seems

Steinbach In essence, all cells that face to the outside and excrete to the outside, tend to reabsorb sodium

Forster During their evolutionary history, since Devonian times, marine fish presumably have been sloughing off their glomeruli in the interest of water conservation I don't see what could be gained 'water-wise' by replacing one leaky membrane with another, that is, discarding the glomerulus in exchange for another non selective membrane, made up in this case of thick epithelial cells

Darrow Can you study kidneys in fish without paying attention to what is happening to the gills? The gills must be exchanging electrolyte quite differently

Berliner That is true, but during a period of maintained elevation of the plasma bicarbonate, plus inhibitors which make the urine alkaline in many other species, the urine remains acid in glomerular fish (dogfish)

Darrow Yes, but hasn't it lost that function because the gill must be doing something?

Berliner Yes, that's probably true

Wallace Does anybody know anything about the choroid plexus? That is the only membrane I can think of which has not been mentioned here What does it do? It only pumps water and ultra filtrate in one direction, doesn't it?

Ussing Couldn't there be active sodium transport, too? The sodium is slightly higher in the secretion, as far as I remember

Wallace Could that be accounted for by the differences in protein concentration?

Ussing Yes, it might

Darrow I should like to ask what the zoologists know about the transport of other ions, that is which ones have transport mechanisms amongst the chloride bicarbonate and potassium?

Ussing I think there are cases of active transport in all of them Sodium, most definitely, but also chloride, at least under certain circumstances, and potassium in the red cell, I don't know how else one could explain the high potassium concentration in the erythrocytes Apparently, the organism knows how to transport all the monovalent ions But how much the mechanisms are used is still a problem

Wallace Is it necessary to invoke chloride transport in the red cells?

Ussing No not in the red cells I was thinking of the epinephrine-stimulated frog skin, and the gastric mucosa

And as I have already mentioned Dr Jorgensen * in our laboratory, has found that live frogs may take up chloride from potassium chloride solutions even when the potential is not high enough to explain the uptake The chloride uptake takes place as an exchange against bicarbonate

Wallace Does the exchange of chloride and bicarbonate involve a simultaneous transfer of potassium?

Ussing No, the potassium is not taken up at all You could use calcium chloride instead, but even though it is present, the chloride uptake would continue under certain circumstances in the form of an exchange against bicarbonate ions

Mudge I should like to ask what examples there are of intracellular electrolytes whose concentration can be explained by a Donnan equilibrium under normal circumstances Are there any instances of this?

Wallace Red cells can

Mudge No, they can't They are full of chloride

Berliner Do you mean the situation Conway postulated for muscle?

Mudge That's right

Wallace The red cell is Conway's ideal cell He tried to make the muscle be like it

Mudge No, the intracellular chloride in the red cell completely upset the calculation

Berliner That's right

*Unpublished data

Wallace No, not necessarily There may be simply more chloride in red cells than in muscle cells Perhaps the muscle cell has so much other anion in it that there is simply no space for chloride Is there little chloride in muscle cell just because there is so much phosphate which is nondiffusible?

Mudge The equation as written states in terms of concentration, that potassium inside, over potassium outside, equals chloride inside, over chloride outside, and so forth and so on For what cells is this true?

Wallace Why isn't it true about red cells? The distribution of anion is best described by a Donnan type of equilibrium

Mudge No, I disagree I don't think it's true for red cells Taking the analyses of human erythrocytes the first two terms in the equation would be

$$\frac{115}{5} = \frac{105}{75} \qquad [10]$$

and arithmetically, that's just not correct

Berliner The Donnan equilibrium applies very well to the distribution of anions inside and outside the red cells, as described by Van Slyke and his co workers (19), but it does not explain the cation distribution

Mudge I know of no normal mammalian cell whose composition is accurately described by the equation It is not valid for kidney, for erythrocytes, or for skeletal muscle Accuracy is supposed to be one of the virtues of mathematics and if an equation is found to be inaccurate, I don't see why we keep using it

Ussing It describes conditions in nerve fibers, according to the Hodgkin group (20) But, of course, those nerves were taken out of the body, and whether they are like Conway's muscle I don't know

Mudge Why do you keep using this equation Dr Darrow?

Darrow I really used the Donnan effects because I wanted to see what Dr Ussing's reaction would be, but it seems to me that the problem is somewhat analogous to a Donnan equilibrium in this sense, that you have diffusible and indiffusible anions and cations in the cell which are in equilibrium with similar ions in extracellular fluids The concentrations of some ions in the cells are controlled by factors other than osmotic and electric forces Now, that isn't what Donnan described, but it is a modification about which we have to think We have to consider how the modifications produced by cellular metabolism alter the osmotic and electrical

equilibrium defined by Donnan The circumstances were peculiar in that the anions which were indiffusible inside the membrane were the only indiffusible ions, and energy was not supplied to the system

Wallace. The sums don't have to be the same?

Darrow No

Wallace The sum of the intracellular cations can be very different from the sum of those in extracellular fluid

Dock We have a lot of protein there

Ussing Wouldn't we be able to explain the known facts if we assumed that although there is in the cells an appreciable amount of potassium that behaves pretty closely according to the Donnan theory, there may be in certain cell types a fraction of potassium that behaves differently In the cells you have studied there may be an appreciable amount of the fraction that does not behave The whole theory need not be wrong because there is a certain fraction that does not behave according to theory

Steinbach If the Donnan equilibrium, as modified by Conway, is examined, and Conway's test for the applicability of his version is taken, which is principally tissue swelling as potassium chloride is substituted for sodium chloride, then a simple answer can be given There are some vertebrate striated muscles that behave well down to a low level of potassium, and vertebrate nerves follow the prediction fairly well Vertebrate smooth muscle and vertebrate cardiac muscle are completely off – they absolutely fail to show the modified Donnan equilibrium at all – and invertebrate muscles in general, more especially the marine invertebrate muscles, nerves and other tissues that have been tested, frequently behave very oddly indeed That, however, does not mean that the Donnan equilibrium is of no use, it would be a mistake to say that What it amounts to is that cells may have a number of active processes which can go on between the inside and the outside of the cell, and the cell is capable of making due allowance for them Certainly no simple formulation works in the majority of the cell types that have been studied

Darrow Perhaps none

Steinbach Perhaps none, but I dont think there is cause for worry.

Lotspeich You are altering our teaching

Steinbach No For example, I disagree violently with respect to mechanisms assumed by Dr Conway, but I must admit that if you assume the mechanism he does, the formulation works beauti-

Wallace No, not necessarily There may be simply more chloride in red cells than in muscle cells Perhaps the muscle cell has so much other anion in it that there is simply no space for chloride Is there little chloride in muscle cell just because there is so much phosphate which is nondiffusible?

Mudge The equation as written states, in terms of concentration, that potassium inside, over potassium outside, equals chloride inside, over chloride outside, and so forth and so on For what cells is this true?

Wallace Why isn't it true about red cells? The distribution of anion is best described by a Donnan type of equilibrium

Mudge No, I disagree I don't think it's true for red cells Taking the analyses of human erythrocytes the first two terms in the equation would be

$$\frac{115}{5} = \frac{105}{75} \qquad [10]$$

and arithmetically, that's just not correct

Berliner The Donnan equilibrium applies very well to the distribution of anions inside and outside the red cells, as described by Van Slyke and his co workers (19), but it does not explain the cation distribution

Mudge I know of no normal mammalian cell whose composition is accurately described by the equation It is not valid for kidney, for erythrocytes, or for skeletal muscle Accuracy is supposed to be one of the virtues of mathematics, and if an equation is found to be inaccurate, I don't see why we keep using it

Ussing It describes conditions in nerve fibers, according to the Hodgkin group (20) But, of course, those nerves were taken out of the body, and whether they are like Conway's muscle I don't know

Mudge Why do you keep using this equation, Dr Darrow?

Darrow I really used the Donnan effects because I wanted to see what Dr Ussing's reaction would be, but it seems to me that the problem is somewhat analogous to a Donnan equilibrium in this sense, that you have diffusible and indiffusible anions and cations in the cell which are in equilibrium with similar ions in extracellular fluids The concentrations of some ions in the cells are controlled by factors other than osmotic and electric forces Now, that isn't what Donnan described, but it is a modification about which we have to think We have to consider how the modifications produced by cellular metabolism alter the osmotic and electrical

equilibrium defined by Donnan The circumstances were peculiar in that the anions which were indiffusible inside the membrane were the only indiffusible ions, and energy was not supplied to the system

Wallace. The sums don't have to be the same?

Darrow. No

Wallace The sum of the intracellular cations can be very different from the sum of those in extracellular fluid

Dock We have a lot of protein there

Ussing Wouldn't we be able to explain the known facts if we assumed that although there is in the cells an appreciable amoun of potassium that behaves pretty closely according to the Donna theory there may be in certain cell types a fraction of potassiun that behaves differently In the cells you have studied there may be an appreciable amount of the fraction that does not behave The whole theory need not be wrong because there is a certain fraction that does not behave according to theory

Steinbach If the Donnan equilibrium as modified by Conway is examined, and Conway's test for the applicability of his version is taken, which is principally tissue swelling as potassium chloride is substituted for sodium chloride then a simple answer can be given There are some vertebrate striated muscles that behave well down to a low level of potassium, and vertebrate nerves follow the prediction fairly well Vertebrate smooth muscle and vertebrate cardiac muscle are completely off – they absolutely fail to show the modified Donnan equilibrium at all – and invertebrate muscles in general, more especially the marine invertebrate muscles, nerves and other tissues that have been tested, frequently behave very oddly indeed That, however, does not mean that the Donnan equilibrium is of no use, it would be a mistake to say that What it amounts to is that cells may have a number of active processes which can go on between the inside and the outside of the cell, and the cell is capable of making due allowance for them Certainly no simple formulation works in the majority of the cell types that have been studied

Darrow Perhaps none

Steinbach Perhaps none, but I don't think there is cause for worry

Lotspeich You are altering our teaching

Steinbach No For example, I disagree violently with respect to mechanisms assumed by Dr Conway, but I must admit that if you assume the mechanism he does, [illegible] ulation works beauti

fully for frog muscle There is nothing wrong with making the assumption of the mechanism if you don't know any more about it

Wallace This turns out to be an active transport of potassium, is that what you mean?

Steinbach No, in the case of the frog muscle, instead of a system impermeable to sodium, we are obviously dealing with an active transport of sodium But thermodynamically, as far as the Donnan equilibrium is concerned, it doesn't care whether the mechanism is a blank wall or a pump It doesn't make any difference

Darrow It is fixed by processes other than simple diffusion

Binkley Are you not saying that we should consider at least three or more processes in explaining the differential distribution of ions? I believe it is commonly taught that in the case of intracellular potassium, for instance, one must consider the Donnan equilibrium, the specific binding of potassium and, finally, invoke a pump to explain all that can t be explained any other way

Darrow I am still bothered Dr Steinbach, by your cells treated with choline You are attributing everything to lack of any sodium to pump when you don't have any diffusion into it

Steinbach Well, as I remarked before, the simple flow of ions into a cell, I think, is a very slow process, and as evidence of that I would point to the extremely long time that it takes at low tem perature to get much sodium in there, so the fact that I don't get much leakage in a two or three-hour period doesn't bother me

Darrow Maybe you are right, but I am not sure

Steinbach As I say, if it bothered me more, and if it really bothers you, I will do the experiment and let it run for twenty four hours and see if I get the sodium substituting for choline, and the pump starting up

Pitts I think that would be an awfully good control experi to show that your cells are really still active muscle cells

Thorn Again, it would be interesting in trying to correl similarities between kidney and intestinal tract, to investig carefully the tandem effect of connecting the kidney and via the ureters The capacity to keep such anastomoses i days with antibiotics might provide a very interesting approach, although we should keep in mind that tandem would be feasible, i e , the bowel action f the kidney, since the reverse procedure would attain

Visscher In the press of the other conversation, to Dr Steinbach's question as to what similari

between the gut and the kidney. It seems to me there are not o the similarities with regard to sodium conservation for the bo but also the quantitatively small, but phenomenally importan changes in the activity of water in the gut, like that in the kidney. The gut does not operate without altering osmotic activity within it. I think we have to look at the gut with a good deal of caution, if we are thinking in terms of a 'macromodel' for some things that may occur in the kidney, and not allow ourselves to be trapped into believing that because there are some coincidences, necessarily the processes are the same. Nevertheless, if we see the same processes occurring in several organs, I think it would be well worth our while to try to find out the mechanisms in the simplest case that we could work with. Perhaps the gut is going to be simpler to analyze as to mechanism than the kidney, because of the greater difficulties in working with a kidney that is not entirely normal under analytical conditions. It probably is true that the greatest difficulty with our perfusion studies is that we cannot be sure just how much deviation from normality we have produced, whereas with the intestine we have an organ that, to be sure is sensitive, but will apparently stand a good deal more abuse than the aglomerular fish kidney.

Lotspeich: The intestine does not seem to be an organ that would be susceptible to such discrete study as the kidney because of the heterogeneity of its cell structure and its function.

Dock: Frog skin is better, you mean?

Lotspeich: Yes.

Visscher: Dr Lotspeich, isn't there heterogeneity of structure in the kidney?

Lotspeich: I am sure there is, but you don't have glands within the organ.

Visscher: That is perfectly true.

Mudge: I should like to emphasize a different aspect of the roblem. While the simplest tissue may offer certain advantages r physiological work, it is quite possible that the most complicated, the most active cells, are better from the biochemical point of view. As an example, consider the problem of cation binding which we have been discussing. There is fair agreement that this phenomenon may have something to do with transport — at least, it is a very useful hypothesis. In the kidney, a very active tissue, 8 per cent of the potassium is "bound," in the liver about 2 per cent, and in the red cell, which has a fairly sluggish transport system, there is no evidence of potassium binding. This surely does not —

mean that potassium is not bound by erythrocytes, it just indicates that not enough is bound to be picked up by our present techniques If only one part in a thousand were bound, it is conceivable that that one part might be very important in transport From the biochemical point of view, some of these reactions are certainly best examined in the most active organs

Ussing I was wondering whether, on a surface basis, the frog skin and the gut aren't working just as fast as the kidney tubule I think it is difficult to calculate

Mudge Yes but one should be able to do it

Ussing It is surprising, really, how much is transported by the gut and the skin

Mudge What does the whole gut reabsorb — a fifth of the total plasma sodium?

Visscher A fifth per ten minutes, perhaps What is the area of the entire gut as compared with the surface area of all the tubules in the kidney? This is a little problem in mathematics which we have to work out first, but we do know that if calculations are made from the flux values which I showed this morning, all the sodium in the plasma of the dog passes through the alimentary tract and is reabsorbed once in, I think, 83 minutes I am not sure that is the exact figure, but it is a fairly short period of time My guess is that the area figures would come out as Dr Ussing suggests, and that it may be going on just about as fast

Mudge Well, I think the gut is all right The thing I don't like is the red cell I think too many studies are extrapolated from red cells to the tissues

Wallace Isn't time a factor to consider? When the electrolyte intake of an individual is suddenly increased, the gastrointestinal tract will handle it, but renal excretion will initially fall short of maintaining balance and the subject will gain weight The kidney will gradually gain the ability to excrete the increment even while the increased intake is maintained I am not sure that this is germane to the argument, but it seems to me that you cannot talk about transfer of electrolytes in a quantitative sense until a steady state is achieved This may take many days

Lotspeich How many liters of fluid does the gut reabsorb per day?

Visscher We have never really obtained a good figure for the total flux for water and all the figures that are given in the text books are values for net absorption which of course are open to great question

Darrow They are handed down from twenty five or thirty years ago

Thorn I should think that the important correlation would be that which occurred during active periods of digestion One would expect a rapid rate of turnover of secretory enzymes, electrolytes, and so forth, in the first two to four hours after the ingestion of food Following this, the over-all flux might fall to a low level during the interval periods without active digestive processes going on

Lotspeich The kidney reabsorbs what — about 140 liters a day?

Visscher Yes The gut would probably not have to do as much in order to have a comparable activity, because I think there would be very much less error As I say, I haven't made the calculations

Dock May the kidney also have active diffusion going on like the gut, in addition to what is filtered? I mean, water can be diffusing through a tubule and back again, we don't have to assume that the tubular cell lets water go only one way

Visscher It has occurred to me that there may be quite a difference between the renal tubule and the gut with respect to the rate at which a pump system is working For example, sodium is being moved from the interior of the tubule to the peritubular space, and back into the blood but what happens as regards movement in the other direction is that there is a certain leakage if you like, and that it may be the leakage that determines how much of a load is put on the active pump mechanism If the leak is more than so much the net urinary excretion is going to increase It may be the control of the leak which determines how much sodium is retained in the body, for example It may be that desoxycorticosterone has an effect on the leakiness of that membrane, rather than having an effect on some carrier mechanism

As I said earlier, I am simply throwing out these suggestions, because I *do not think* we *have any* right to be dogmatic at our present stage of knowledge about this problem I don't think we have enough information to say that only one particular explanation will account for this, that, or the other, mechanism I think we must keep an open mind, and look at all the possible solutions until we have proof that one or the other is either impossible or *inevitable* There are so *many conclusions* that are possible, but not inevitable, at the present time and until we know more, I don't think we should exclude any of the possible mechanisms

Pitts I wonder, in connection with your discussion of flux of water, and of sodium and potassium in both directions through the

renal tubule where it puts us with respect to flux of the second most diffusible component in the body namely urea with relation to the excretory processes involved in the excretion of urea?

Visscher You mean how could a membrane be impermeable for urea and not for sodium ion?

Pitts Yes basically that

Visscher Well I don t know enough about the hydrated molecular diameter of urea for one thing to be able to answer that question How about that Dr Ussing?

Ussing Actually I could not say There is always the question of charge in the membrane In a membrane phase which was negatively charged the sodium ion would pass more easily whereas an uncharged one might not go so fast

Visscher The capillary membrane is such a leaky membrane compared with the structures we are talking about

Dock But for the relative sizes Pappenheimer s data are available

Berliner He has estimates of the effective radius based on diffusion data

Ussing That does not relate to charge effects

Dock In one human disease reabsorption of urea and glucose from the gut are interfered with In sprue a patient can t absorb dextrose from his upper gastrointestinal tract and he doesn t absorb urea properly either for some strange reason

Visscher Well I might have said that if you place urea in salt solutions at approximately blood concentrations of urea while sodium and chloride reabsorption are going on there is a concentration of urea in the gut That bears on your point

Pitts So then the gut isn t really so nonselective

Visscher No I don t think it is It is absorbing sodium chloride against concentration gradients and leaving urea I don t know enough about the physicochemical characteristics of urea in solutions to give you any really intelligent answer to your question

REFERENCES

1 OLIVER J and SHEVKY E Experimental nephritis in the frog perfusion methods of testing the kidney by dissociation of its functions *J Exper Med* 53 *763 (1931)*

2 SCHMIDT NIELSEN B and FORSTER R P The effect of some ecological factors on renal function in the frog (*Rana catesbeiana*) *Bull Mt Desert Isle Biol Lab* (In press)

3 FORSTER R P The use of inulin and creatinine as glomerular filtrate measuring substances in the frog *J Cell & Comp Physiol* 12 *213 (1938)*

4 SAWYER W H and SAWYER M K Adaptive responses to neurohypophyseal fractions in vertebrates *Physiol Zool* 25, 84 (1952)
5 HOSHIKO T, SWANSON R E and VISSCHER M B Movement of D_2O and Na^{24} across the frog renal tubule in the direction of capillary to tubular lumen *Federation Proc* 11, 73 (1952)
6 FORSTER R P The effect of epinephrine upon frog renal hemodynamics in the intact animal *Am J Physiol* 140 221 (1943)
7 MARSHALL E K JR and SMITH H W The glomerular development of the vertebrate kidney in relation to habitat *Biol Bull* 59 135 (1930)
8 SMITH H W The absorption and excretion of water and salts by marine teleosts *Am J Physiol* 93, 480 (1930)
9 GRAFFLIN A L Urine flow and diuresis in marine teleosts *Am J Physiol* 97, 606 (1931)
10 SOBOTKA H and KANN S Carbonic anhydrase in fishes and invertebrates *J Cell & Comp Physiol* 17 341 (1941)
11 WILBRANDT W Electrical potential differences across the wall of kidney tubules of Necturus *J Cell & Comp Physiol* 11 425 (1938)
12 EMERSON K JR KAHN S S and JENKINS D The role of the gastrointestinal tract in the adaptation of the body to the prevention of sodium depletion by cation exchange resins *Ann N Y Acad Sc* (In press)
13 ELKINTON J R SQUIRES R D and KLINGENSMITH W C JR Cation exchange resin in the treatment of congestive heart failure I Electrolyte exchanges during initial periods of resin therapy *Circulation* 5, 747 (1952)
14 RAMSAY J A Osmotic regulation in mosquito larvae *J Exper Biol* 27 145 (1950)
15 ——— The site of formation of hypotonic urine in the nephridium of Lumbricus *J Exper Biol* 26 65 (1949)
16 ROBERTSON J D Ionic regulation in some marine invertebrates *J Exper Biol* 26 182 (1949)
17 RAMSAY J A The excretion of sodium and potassium by the Malpighian tubules of Rhodnius *J Exper Biol* 29 110 (1952)
18 HOYLE G High blood potassium in insects in relation to nerve conduction *Nature* 169, 281 (1952)
19 VAN SLYKE D D WU H and MCLEAN F C Studies of gas and electrolyte equilibria in the blood V Factors controlling the electrolyte and water distribution in the blood *J Biol Chem* 56 765 (1923)
20 HODGKIN A L The ionic basis of electrical activity in nerve and muscle *Biological Rev* 26, 339 (1951)